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ANNUAL RESEARCH PROGRESS REPORT

(FY 2008)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58202

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ANNUAL RESEARCH PROGRESS REPORT

(FY 2008)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

**UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA**

GRAND FORKS, NORTH DAKOTA 58202

NUTRITIONAL DETERMINANTS OF HEALTH

MANAGEMENT UNIT

5450-010-00

Project Number: 5450-51000-038-00D Accession: 0408766 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: DAVID M KLURFELD Prin Invs: WILLIAM T JOHNSON

Start Date: 07/21/2004 Term Date: 04/30/2009

National Programs: 107 N Human Nutrition

Title: DIETARY COPPER REQUIREMENTS FOR OPTIMAL CARDIOVASCULAR FUNCTION AND HEALTH

Period Covered From: 10 / 2007 To: 9 / 2008 Final Report? No

Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Overall, to determine, using animal models, whether copper (Cu) intakes consistent with those observed in humans can adequately support cardiovascular functions. To develop a strategy for assessment of marginal copper deficiency in animals; to use this strategy to determine biomarkers of copper status that are suitable for assessment of marginal status in humans. To determine the contribution of oxygen- and nitrogen-derived reactive species to the cardiomyopathy (metabolic, contractile) induced by Cu deficiency, and the dietary intakes at which this pathology occurs. To determine whether low Cu intakes consistent with those observed in humans can impair nitric oxide-dependent control of blood vessels and blood pressure regulation. To determine whether the oxidative stress induced by Cu deficiency affects homocysteine metabolism and, thereby, cardiovascular function, and whether such effects influence nitric oxide-dependent signal transduction and/or other mechanisms that affect atherosclerosis. To determine whether marginal Zn deficiency can exacerbate or unmask cardiovascular effects of sub-optimal Cu status by virtue of its role in oxidative/nitrosative metabolism.

1b. Approach (from AD-416)

Laboratory animals of varying ages and, in some cases, varying genetic makeup will be fed diets containing copper in severely deficient, marginally deficient or adequate amounts for varying periods of time. Based on comparisons to reliable invasive markers of copper status, non-invasive biomarkers will be tested for validity in assessing marginal copper deficiency. Tests of cardiovascular function and examinations of mechanism of depressed function will be made over ranges of copper status varying from severely-deficient to adequate. Cardiovascular functional measurements include heart contractile function, cardiac mitochondrial respiration and respiratory complex activity, blood vessel contractility and blood pressure. Atherosclerosis will be assessed by microscopic observation of blood vessels. Examination of mechanisms of depressed function will focus on the effects of oxidative stress and altered nitric oxide metabolism known to occur in copper deficiency. Such examination will include assessment of oxidative/nitrosative damage, altered nitric oxide signaling and altered homocysteine metabolism and will extend to measurement of transcription factors, mRNA and enzymes that influence and are influenced by oxidative stress and nitric oxide signaling.

2. Milestones for FY2008

1. Determine nature of effect of altered nitric oxide (NO) on coronary vessels--This milestone was substantially met. It could not be fully met because a critical vacancy prevented examination of coronary vessels. The experiments for this

Project Number: 5450-51000-038-00D

Accession: 0408766

FY: 2008

milestone were completed using lung and peripheral blood vessels.
Milestone Substantially Met

2. Determine role of hemeoxygenase in atherosclerotic effects of copper deficiency.
Milestone Not Met

Critical vacancy (quantitative or qualitative deficiency in personnel

3. Progress Report

To determine the relative contributions of prenatal and postnatal contributions of maternal copper nutrition to the developmental expression of mitochondrial cytochrome c oxidase in offspring, the activity of cytochrome c oxidase and subunit content was measured in the hearts of offspring of copper-deficient dams that were cross fostered to copper-adequate dams at birth and in the hearts of the offspring of copper-adequate dams that were cross fostered to copper-deficient dams. The study was successfully completed and showed that prenatal copper intake by the dam was the major contributor to impaired postnatal expression in the heart mitochondria of the offspring. This finding is now being applied in the planning of another experiment to determine if prenatal gene expression is altered by low maternal copper intake during pregnancy in a manner that perpetuates impaired cardiac mitochondrial function in adult offspring. (NP107, Component 4: Nutrient Requirements)

One of the milestones for 2009 is to identify oxidized and nitrated proteins in cardiac mitochondria of copper-deficient dams. A study measuring protein oxidation and reactive oxygen production in cardiac mitochondria from severely copper-deficient, marginally copper-deficient, and control rats was initiated. (NP107, Component 4: Nutrient Requirements)

Trace element analyses of samples from the experiment showing that a moderate zinc deficiency did not exacerbate signs of oxidative stress in marginally copper deficient rats were completed. The moderate zinc deficiency decreased urinary magnesium excretion and increased kidney and tibia magnesium concentrations, which indicates increased magnesium retention. (NP107, Component 4: Nutrient Requirements)

4. Accomplishments:

- 1 Copper supplementation decreases the size and increases the number of cardiomyocytes in hearts enlarged by copper deficiency.
In experimental rodents, copper deficiency causes cardiac enlargement that can be reversed by restoring normal copper status. However, the mechanism through which copper supplementation reverses cardiac enlargement is not completely understood. In copper-deficient mice, supplementation with the normal dietary copper reversed cardiac enlargement by directly decreasing the size of cardiomyocytes and also by promoting replication of hypertrophied cardiomyocytes with reduced size. **IMPACT:** This finding indicates that normal copper intake may allow damaged, enlarged cardiomyocytes to undergo replication in order to repair damage and normalize their size and function. Cardiac enlargement and heart failure are a major cause of disability and death in humans. This study indicates that dietary copper requirements may increase in people with certain types of heart disease associated with cardiac enlargement and that supplemental copper may be beneficial for improving cardiac function in these people. (NP107, Component 4: Nutrient Requirements)
- 2 Low copper intake during pregnancy alters vascular relaxation in first and second generation offspring.
Previous studies have shown that copper deficiency attenuates vascular relaxation in

Project Number: 5450-51000-038-00D

Accession: 0408766

FY: 2008

rats. However, it is not known whether copper deficiency during pregnancy and lactation affects vascular responses in offspring. It was found that first generation female offspring of marginally copper-deficient dams experienced enhanced vascular relaxation in response to vasodilators. In contrast, second generation female offspring whose dams were of the first generation from the marginally copper-deficient dams experienced reduced vascular relaxation in response to vasodilators. The vascular responses of first and second generation male offspring were not affected by the copper status of the original dams. These findings indicate that copper deficiency during pregnancy and lactation have a direct effect on vascular responses in first generation offspring that is perpetuated in second generation offspring in a gender-dependent manner. IMPACT: Dietary surveys indicate that pregnant women often do not meet the current recommended daily intake for dietary Cu. This finding indicates that moderately low Cu intake by pregnant women may alter the programmed development of the vasculature in a manner that increases the risk of heart disease in their children and grandchildren because of an altered response of blood vessels to relaxation stimuli. (NP107, Component 4: Nutrient Requirements)

3 Dietary copper and selenium interact to affect the concentration of plasma homocysteine.

Previously we reported that copper deficiency decreased plasma homocysteine and increased plasma glutathione in rats. Other work that we have done shows that selenium deficiency also decreases plasma homocysteine and increases plasma glutathione in rats. We suggested a common mechanism in each deficiency - an up-regulation in the enzyme that makes glutathione from cysteine, a metabolite of homocysteine. Our findings showed that a combined deficiency of both copper and selenium did result in significantly lower plasma homocysteine and significantly elevated plasma glutathione. Studies are ongoing to determine whether marginal deficiencies have synergistic effects on plasma homocysteine and glutathione. IMPACT: Plasma homocysteine is a biomarker of cardiovascular disease. Understanding dietary factors that affect the plasma concentration of homocysteine are needed to validate its impact as a biomarker. (NP107, Component 4: Nutrient Requirements)

4 Moderate zinc deficiency influences magnesium metabolism in rats

Severe zinc deficiency induces signs of inflammatory and oxidative stress that often are not seen in marginally zinc-deficient experimental animals, which suggests compensatory mechanisms may occur that overcomes mild oxidative and inflammatory stress induced by a moderate zinc deficiency. An experiment at Grand Forks, ND, with rats showed that a moderate zinc deficiency decreased urinary magnesium excretion and increased kidney and tibia magnesium concentrations, which indicated increased magnesium retention. Because magnesium has anti-inflammatory and anti-oxidant stress actions, increased magnesium retention and utilization may be involved in overcoming the oxidant and inflammatory stress of moderate zinc deficiency. IMPACT: Further studies are needed to determine whether combined moderate zinc and magnesium deficiencies, each that commonly occur, is a factor that results in increased oxidative and inflammatory stress that can lead to cardiovascular disease. (NP107, Component 4: Nutrient Requirements)

5. Significant Activities that Support Special Target Populations

None.

Project Number: 5450-51000-038-00D

Accession: 0408766

FY: 2008

6. Technology Transfer

- 0 Number of New CRADAs
- 0 Number of Active CRADAs
- 0 Number of New MTAs (providing only)
- 0 Number of Invention Disclosures Submitted
- 0 Number of Patent Applications Filed
- 0 Number of New Germplasm Releases
- 0 Number of new commercial licenses granted
- 0 Number of web sites managed
- 4 Number of non-peer reviewed presentations and proceedings
- 2 Number of newspaper articles and other presentations for non-science audiences
- 0 Number of Other Technology

Non Peer Reviewed Presentations and Proceedings Details:

- 01 **Description:** Technology: Influence of low copper intake during pregnancy and lactation on cardiovascular outcomes across generations
Transfer: Oral presentation (Johnson, W.T., Anderson, C.M. Prenatal copper deficiency in rat dams causes persistent reduction in nuclear-encoded cytochrome c oxidase subunits in cardiac mitochondria of the first generation, FASEB J 22:443.2) and poster presentation (Anderson, C.M., Johnson, W.T. Marginal copper deficiency impairs endothelium-dependent relaxation responses across two generations, FASEB J 22:695.1) at the Experimental Biology 2008 meeting, San Diego, CA, April 5-9, 2008.
Customer/User: Other scientists, health professionals
Impact/Outcome: Expand knowledge regarding the importance of adequate copper nutrition during pregnancy and provide ideas for further research into the relationship between dietary copper during pregnancy and cardiovascular disease in offspring.
- 02 **Description:** Technology: Dietary selenium and copper interact to affect homocysteine metabolism in rats
Transfer: Poster presentation given at Experimental Biology annual meeting (Uthus, E.O., Ross, S.A. Dietary selenium (Se) and copper (Cu) interact to affect homocysteine metabolism in rats, FASEB J 22:696.3). Experimental Biology 2008 meeting, San Diego, CA, April 5-9, 2008.
Customer/User: Other scientists, medical professionals
Impact/outcome: Expand understanding of the role of copper in cardiovascular health.
- 03 **Description:** Technology: Copper and zinc needs for cardiovascular health.
Transfer: Poster presentation (Nielsen F.H., A combined marginal deficiency of copper and zinc does not exacerbate oxidant stress associated with copper or zinc deficiency, FASEB J 22:1103.1) given at Experimental Biology 2008 in San Diego, CA, April 2008
Customer/User: Other scientists, nutrition professionals
Impact/Outcome: Reduce cardiovascular disease by encouraging the consumption of both copper and zinc at RDA amounts because these elements by themselves affect indicators of oxidative and/or inflammatory stress in ways associated with an increased risk for cardiovascular disease.

Project Number: 5450-51000-038-00D

Accession: 0408766

FY: 2008

04 **Description:** Technology: Copper, zinc and magnesium needs for cardiovascular health.
Transfer: Oral presentation (Nielsen F.H., Zinc, copper, magnesium and boron benefit human health: implications for meat and egg products to supply greater amounts of these minerals to consumers) to a group of food industry nutrition professionals attending the World Poultry Conference in Niagara Falls, Canada, July 24, 2008.
Customer/User: Food industry representatives, nutrition professionals, consumers
Impact/Outcome: Reduce cardiovascular disease by encouraging the food industry to provide products that increase the intakes of copper, zinc and magnesium.

Newspaper Articles and Other Presentations for Non Science Audiences Details:

- 01 **Description:** Technology: Understanding roles for trace element nutrients in cardiovascular disease
Transfer: Newspaper, Grand Forks Herald, August 2007
Customer/User: General public
Impact/outcome: Provide information for better cardiovascular health through nutrition
- 02 **Description:** Technology: Understanding roles for trace element nutrients in cardiovascular disease
Transfer: Newspaper, Grand Forks Herald, December 2007
Customer/User: General public
Impact/outcome: Provide information for better cardiovascular health through nutrition

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

1. Johnson, W.T., Johnson, L.K. 2008. Copper Deficiency Inhibits CA2+-Induced Swelling in rat Cardiac Mitochondria. Journal of Nutritional Biochemistry.doi.10.1016/j.jnutbio.2008.02.009

000022009

2. Johnson, W.T., Anderson, C.M. 2008. Cardiac Cytochrome c Oxidase Activity and Contents of Submits 1 and 4 are Altered in Offspring by Low Prenatal Intake by Rat Dams. Journal of Nutrition. 138:1269-1273

000021795

3. Kang, Y.J., Jiang, Y., Saari, J.T. 2007. Changes in copper and zinc status and response to dietary copper deficiency in metallothionein-overexpressing transgenic mouse heart. Journal of Nutritional Biochemistry. 18(11):714-718.

000013330

4. Klevay, L.M., Aladjem, M., Bogden, J.D., Sandstead, H.H., Kemp, F.W., Li, W., Skurnick, J., Aviv, A. 2007. Renal and gastrointestinal potassium excretion in humans: new insight based on new data and review and analysis of published studies. Journal of the American College of Nutrition. 26(2):103-110.

000016547

Project Number: 5450-51000-039-00D Accession: 0409965 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: CURTISS HUNT

Start Date: 08/01/2005 Term Date: 04/30/2009

National Programs: 107 N Human Nutrition

Title: MINERAL INTAKES FOR OPTIMAL BONE DEVELOPMENT AND HEALTH

Period Covered From: 10 / 2007 To: 9 / 2008 Final Report? No

Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Enhance the quality of life through establishing mineral intakes that support optimal bone health. Specifically, determine the amount of dietary calcium needed to maximize calcium retention and minimize bone resorption in postmenopausal women; determine the extent to which dietary protein, specific mineral elements (zinc, copper, magnesium, and boron) and prebiotics (inulin) interact with dietary calcium to affect bone metabolism.

1b. Approach (from AD-416)

Studies will utilize human subjects and animal models. Human studies will use the Mobile Nutrition Research Laboratory, the in-house Community Studies Unit, and the Metabolic Research Unit to conduct epidemiological supplementation, fortification, and controlled feeding experiments, respectively. In each case, subjects will be fed diets containing marginal to high amounts of mineral elements to determine how specific minerals, and interactions among them, affect bone structure (as determined by light microscopy, biomechanical assessment, and densitometry) and biomarkers [urinary deoxypyridinoline, hemoglobin Alc, and C-reactive protein]). When relevant, the modifying influence of selected hormonal (e.g., estrogen deficiency) or diet compositional (e.g., inulin) factors will be examined.

2. Milestones for FY2008

1. Report on study on estimating the Ca requirement by titration; substitution of meta-analysis of existing human calcium balance data.
Milestone Fully Met
2. Recruit and conduct community-based trial for acid-base balance and calcium retention.
Milestone Fully Met
3. Conduct the third of a three year study on bone health and copper and zinc supplementation.
Milestone Fully Met
4. Initiate in vitro studies for determining the role of Zn and Zn transporter, ZIP1, in the osteoclastogenesis process.
Milestone Substantially Met
5. Complete post-growth phase of boron essentiality study.
Milestone Fully Met

Project Number: 5450-51000-039-00D

Accession: 0409965

FY: 2008

6. Report on study on whether dietary boron improves calcium absorption
Milestone Fully Met

7. Initiate studies to determine whether polyphenols in beans slow age-related bone loss
Milestone Fully Met

3. Progress Report

To determine whether a high acid load from high-meat protein diet, coupled with consumption of other foods that increased the acid load, influences calcium metabolism, post-menopausal women's calcium absorption was measured from menus planned to provide high-meat protein and other high acid load foods (cereal/grains) or low-meat protein and low acid load foods (fruits and vegetables). The dietary intervention is completed and chemical analyses are in process. (NP107, Component 4: Nutrient Requirements)

The effect of graded levels of dietary calcium on bone mass, morphology, and biomechanical properties was assessed with the long bones (tibias and femurs) of female rats after consuming experimental diets for 13 wk. All aspects of the study were completed successfully. (NP107, Component 4: Nutrient Requirements)

To determine whether consumption of a diet containing adequate boron during pregnancy is important in embryological development and growth of bones, late gestation rat fetuses were taken from mothers fed low or adequate amounts of boron and analyzed for bone structure development. The dietary intervention and analyses of bone structure were completed successfully. (NP107, Component 4: Nutrient Requirements)

To determine the function of zinc and zinc transporter, ZIP1, in the osteoclastogenesis process, a cell culture model of bone-resorbing cells (osteoclasts) was used to measure osteoclast activity and expression of ZIP1 during zinc depletion or supplementation. Analyses of cell proliferation, apoptosis, osteoclast formation, and gene expression were completed successfully. (NP107, Component 4: Nutrient Requirements)

To determine whether dietary boron has positive effects on bone health through influencing utilization of S-adenosylmethionine, rats were fed boron-deficient or -adequate diets with different fat sources that affect membrane composition and function. The dietary intervention and biochemical analyses were completed successfully. (NP107, Component 4: Nutrient Requirements)

To determine whether natural substances in beans slow age-related bone loss, bone marrow cells were extracted from older mice fed supplements of bean hull extract containing high amount of polyphenols and cultured to determine the effect of the treatment on the function of osteoblasts and osteoclasts. The biochemical analyses were completed successfully and a follow up experiment was initiated to determine whether long-term bean hull extract supplementation affects bone structure of long bones in the mouse model. (NP107, Component 4: Nutrient Requirements)

4. Accomplishments:

- 1 High meat protein diet with high potential acid load does not impair calcium retention: High protein intake has been considered a risk factor for calcium loss, bone loss and osteoporosis that may be further compounded by a greater renal acid load when emphasizing grain-based foods rather than fruits and vegetables. ARS scientists

Project Number: 5450-51000-039-00D

Accession: 0409965

FY: 2008

at Grand Forks, ND, measured dietary calcium retention in healthy post-menopausal women consuming diets that were low in meat protein and potential acid load or high in meat protein and acid load. Dietary calcium retention was not impaired by a diet high in meat protein and acid load. These results provide evidence useful for developing dietary recommendations for meat protein intake to maintain healthy bones. (NP107, Component 4: Nutrient Requirements)

- 2 The calcium requirement for optimal bone health in the rat is similar whether based on bone mass, structure, or biomechanical strength criteria: Calcium supplementation increases bone density but the increase is small and the impact on bone strength and fracture risk is uncertain. ARS scientists at Grand Forks, ND investigated whether dietary calcium requirements differ for optimizing bone mass, structure, or biomechanical strength in rats. Bone mass, structure and strength all optimized with the same amount of dietary calcium (~2.5 grams of calcium per kilogram of diet), indicating that bone breaking strength and structural properties are not improved by increasing dietary calcium intakes beyond those needed to maximize bone density. These animal results can help in comprehending human calcium requirements, as human measurements are commonly limited to bone density. (NP107, Component 4: Nutrient Requirements)
- 3 Bone loss caused by calcium deficiency during adolescence in the rat can be restored by subsequent adequate calcium intake: Adequate dietary calcium intake is required to achieve full accretion of bone. However, there is little evidence to address the question of whether inadequate calcium intake before sexual maturity can be corrected by calcium repletion afterwards. ARS scientists at Grand Forks, ND, determined that the harmful effects of calcium deficiency on bone structure and strength in female rats can be reversed by switching to proper dietary calcium intakes after sexual maturity. The results indicate that calcium repletion early in adulthood may be an opportune time to correct damage to bones inflicted by poor calcium nutrition during adolescence. These animal results can help in comprehending human calcium requirements, especially related to early adulthood. (NP107, Component 4: Nutrient Requirements)
- 4 Boron is bioactive through modulating the utilization or formation of S-adenosylmethionine needed for numerous biochemical processes: Boron in nutritional amounts has several beneficial effects on bone health but a defined mechanism of action for its bioactivity is lacking. Boron binds tightly to S-adenosylmethionine (SAM), a natural compound in the body that gives rise to homocysteine. Poor control of homocysteine has been implicated in increasing the risk for osteoporosis and bone fractures. ARS scientists at Grand Forks, ND, showed that boron deprivation decreases liver SAM and increased plasma homocysteine in rats, which suggests boron is bioactive through modulating the formation or utilization of S-adenosylmethionine. Increased intakes of boron through consuming foods such as fruits, vegetables, and pulses may reduce the risk for osteoporosis and bone fractures. (NP107, Component 4: Nutrient Requirements)

5. Significant Activities that Support Special Target Populations

None

Project Number: 5450-51000-039-00D

Accession: 0409965

FY: 2008

6. Technology Transfer

- 0 Number of New CRADAs
- 0 Number of Active CRADAs
- 0 Number of New MTAs (providing only)
- 0 Number of Invention Disclosures Submitted
- 0 Number of Patent Applications Filed
- 0 Number of New Germplasm Releases
- 0 Number of new commercial licenses granted
- 0 Number of web sites managed
- 2 Number of non-peer reviewed presentations and proceedings
- 0 Number of newspaper articles and other presentations for non-science audiences
- 0 Number of Other Technology

Non Peer Reviewed Presentations and Proceedings Details:

- 01 **Description:** Technology: Nutritional benefits of flaxseed (boron, magnesium, copper, and omega-3 fatty acids)
Transfer: Invited oral presentation given at the short course
Flaxseed: Adding Functional Food Value (Nielsen, F.H., Nutritional Benefits of Flaxseed, Northern Crops Institute, Fargo, ND, March 26, 2008).
Customer/User: Food industry representatives, nutrition professionals, other scientists, consumers
Impact: Promote health by increasing the consumption of flaxseed in reasonable amounts, which results in intakes of boron, magnesium, copper and omega-3 fatty acids that are beneficial or substantially meet the Food and Nutrition Board's Estimated Average Requirements (EAR) for humans.
- 02 **Description:** Technology: Boron in the typical diet: possible essential roles in human and animal physiology
Transfer: Invited oral presentation given at the 19th annual scientific meeting of the Japan Society for Biomedical Research on Trace Elements (Hunt, C.D., Boron in the typical diet: possible essential roles in human and animal physiology, Tokyo, Japan, July 3, 2008).
Customer/User: Food industry representatives, nutrition professionals, other scientists, consumers
Impact: The session was attended by ~300, and was videotaped for classroom presentation, expanding opportunities for further research to identify the mechanisms responsible for the bone health promoting properties of dietary boron.

7. International Cooperation / Collaboration

01 ENGLAND

King's College in London, England - "Importance of Dietary Silicon for Bone Formation and Maintenance" (under terminated IMOU agreement):
Completed the final step (publication in press). This collaboration found that silicon deprivation in rats did not affect bone quality, but inhibited bone growth plate closure, and increased longitudinal growth. Rats actively maintain bone and body silicon via urinary conservation.

Project Number: 5450-51000-039-00D

Accession: 0409965

FY: 2008

02 ARGENTINA

University of Buenos Aires, Argentina - "Histologic, Histomorphic, and Biochemical Assessment of the Possible Augmentation of Bone Growth on Dental and Bone Implants by Silicon and Boron" (Extramural agreement No. 58-5450-3-F094)

The research, in collaboration with scientists, found that an adequate intake of silicon is needed for alveolar bone formation in rats. The research is complete, but publication of findings has not been completed.

03 ARGENTINA

Enhance the quality of life through establishing mineral intakes that support optimal bone and joint health. Specifically, to confirm that boron is bioactive in osteogenesis and thus promotes bone growth and remodeling; and to establish dietary recommendations for boron that promote bone repair and maintains bone health. This project is under cooperative agreement 5450-51000-039-05N.

Scientific Publications:

Log 115

1. Cao, J.J., Kurimoto, P., Boudignon, B., Rosen, C., Lima, F., Halloran, B.P. 2007. Aging impairs IGF-1 receptor activation and induces skeletal resistance to IGF-1. Journal of Bone and Mineral Research. 22(8):1271-1279. 000021157
2. Gorustovich, A.A., Steimetz, T., Nielsen, F.H., Guglielmotti, M.B. 2008. A histomorphometric study of alveolar bone modeling and remodeling in mice fed a boron-deficient diet. Archives of Oral Biology. 53(7):677:682. 000021348
3. Gorustovich, A.A., Steimetz, T., Nielsen, F.H., Guglielmotti, M.B. 2008. Histomorphometric Study of Alveolar Bone Healing in Rats Fed a Boron-Deficient Diet. The Anatomical Record. 291:441-447. 000021303
4. Hunt, C., Johnson, L.K. 2007. Calcium requirement: new estimations for men and women by cross-sectional statistical analyses of calcium balance data from metabolic studies. American Journal of Clinical Nutrition. 86:1054-63. 000020604
5. Hunt, J.R., Hunt, C., Zito, C.A., Idso, J.P., Johnson, L.K. 2008. Calcium requirements of growing rats based on bone mass, structure, or biomechanical strength are similar. Journal of Nutrition. 138:1462-1468. 000022433
6. Klein, C.J., Nielsen, F.H., Moser-Veillon, P.B. 2008. Trace Element Loss in Urine and Effluent Following Traumatic Injury. Journal of Parenteral and Enteral Nutrition. 32(2):129-139. 000021360
7. Nielsen, F.H. 2008. A novel silicon complex is as effective as sodium metasilicate in enhancing the collagen-induced inflammatory response of silicon-deprived rats. Journal of Trace Elements in Medicine and Biology. 22:39-49. 000020063
8. Nielsen, F.H. 2008. Is Boron Nutritionally Relevant?. Nutrition Reviews. 66(4):183-191. 000021789
9. Turner, K.K., Nielsen, B.D., O'Connor-Robison, C.I., Nielsen, F.H., Orth, M.W. 2008. Tissue response to a supplement high in aluminum and silicon. Biological Trace Element Research. 121(2):134-148. 000022205
10. Turner, K.K., Nielsen, B.D., O'Connor-Robison, C.I., Rosenstein, D.S., Marks, B.P., Nielsen, F.H., Orth, M.W. 2008. Sodium zeolite A supplementation and its impact on the skeleton of dairy calves. Biological Trace Element Research. 121(2):149-159. 000022205

Approved: MCGUIRE MICHAEL R

Date: 09/17/2008

Project Number: 5450-51000-039-05N Accession: 0407993 FY: 2008
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: FORREST H NIELSEN
Start Date: 03/01/2004 Term Date: 02/28/2009

National Programs: 107 N Human Nutrition

Title: HISTOMORPHOMETRIC AND BIOCHEMICAL ASSESSMENT OF THE POSSIBLE AUGMENTATION OF BONE
HEALING AND REMODELING BY BORON

Period Covered From: 10 / 2007 To: 9 / 2008 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0038F

Organization Name: UNIVERSITY OF SALTA

Progress and Outcomes:

1a. Objectives (from AD-416)

Enhance the quality of life through establishing mineral intakes that support optimal bone and joint health. Specifically, to confirm that boron is bioactive in osteogenesis and thus promotes bone growth and remodeling; and to establish dietary recommendations for boron that promote bone repair and maintains bone health.

1b. Approach (from AD-416)

Studies will use a mouse model of bone repair after injury. Mice will be fed boron-deficient and adequate diets. After about 5 weeks, surgical procedures will be performed for the purpose of evaluating peri-implant bone healing of the tibia and mandibular bone remodeling upon tooth extraction. Thirty days after the surgical procedures, the tibias and mandibles will be collected for histologic and histomorphometric examination. Tissues and plasma will be collected for the determination of indicators of bone formation and remodeling.

3. Progress Report

This report serves to document research conducted under a Non-Funded Cooperative Agreement between ARS and the National University of Salta, Argentina. Additional details of research can be found in the report of the parent project 5450-51000-039-00D, Mineral Intakes for Optimal Bone Development and Health

The purpose of this research is to determine whether boron is bioactive in bone formation and thus promotes bone growth and remodeling after tooth extraction. The experimental portion of the research has been completed. The research resulted in two peer-reviewed publications, which are listed in the report for the parent project. The reports showed that boron deprivation compared to nutritional intakes of boron resulted in impaired alveolar bone formation without tooth extraction and alveolar bone healing after tooth extraction in experimental animals because of reduced osteogenesis (bone formation). The findings indicate that boron is required for optimal osteoblast (bone-forming cell) activity, and thus boron is beneficial for bone formation and strength, and low boron status impairs bone healing after injury.

The ADODR monitored research activities through email and personal contact at a scientific meeting, and participated in the writing of the publications through email.

4. Accomplishments:

Project Number: 5450-51000-039-05N

Accession: 0407993

FY: 2008

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/23/2008

Project Number: 5450-51000-039-06S Accession: 0408592 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: CURTISS HUNT

Start Date: 07/16/2004 Term Date: 09/30/2008

National Programs: 107 N Human Nutrition

Title: THE NUTRITIONAL ROLE OF BORON IN THE PREVENTION OF DIABETES

Period Covered From: 10 / 2007 To: 9 / 2008 Final Report? No

Terminate in Two Months? No

Agreement Number: 58-5450-4-0366

Organization Name: NORTH DAKOTA STATE UNIVERSITY

Progress and Outcomes:

1a. Objectives (from AD-416)

To contribute to basic nutrition research on the metabolic roles of boron, particularly as it may affect insulin binding/function.

1b. Approach (from AD-416)

Nutritional requirements and dietary recommendations for the trace mineral boron will be investigated in cell culture and animal models and humans, through application of dietary boron deprivation, supplementation, and repletion. The human studies will be conducted with appropriate review and approval by the respective Institutional Review Boards used by the University and ARS.

3. Progress Report

This report serves to document research conducted under a specific cooperative agreement between ARS and North Dakota State University. Additional details of research can be found in the report of the parent project 5450-51000-039-00D, Mineral Intakes for Optimal Bone Development and Health

To better define the mechanism by which dietary boron decreased plasma insulin levels and increased insulin sensitivity, we have successfully conducted further analysis of muscle insulin receptor concentrations in an animal study to extent our previous findings. The findings were summarized in the parent CRIS. ADODR monitoring activities to evaluate research progress included electronic mail correspondence.

4. Accomplishments:

7. International Cooperation / Collaboration

Project Number: 5450-51000-039-07T Accession: 0408848 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: FORREST H NIELSEN

Start Date: 07/01/2004 Term Date: 04/30/2009

National Programs: 107 N Human Nutrition

Title: EFFECT OF ARGININE SILICATE INOSITOL COMPLEX ON BONE AND JOINT HEALTH

Period Covered From: 10/2007 To: 9 / 2008 Final Report? No
Terminate in Two Months? No

Agreement Number: 04-5450-4-0415

Organization Name: NUTRITION 21, INC.

Progress and Outcomes:

1a. Objectives (from AD-416)

To determine whether silicon as an arginine silicate inositol complex is useful in overcoming the lack of dietary silicon that may lead to pathological changes in bone or connective tissue including bone cartilage (e.g., osteoarthritis) and thus result in bone erosion or loss.

1b. Approach (from AD-416)

Dark agouti rats that are especially susceptible to chronic and severe forms of autoimmune arthritis will be injected with type II collagen to induce arthritis after being fed silicon-deficient and silicon supplemented (as arginine silicate inositol complex) for 5 weeks. The development of arthritis will be evaluated by a macroscopic scoring system and joint swelling measurements. During arthritis development urinary bone breakdown and plasma inflammatory variables will be determined. Five weeks after injection of collagen II the animals will be killed for the determination of blood and bone variables associated with arthritis development and bone loss. Bones will be examined histologically to evaluate the effect of dietary silicon on cartilage erosion and bone resorption.

3. Progress Report

This report serves to document research conducted under a Trust Fund Cooperative Agreement between ARS and Nutrition 21, Inc., Purchase, NY. Additional details of research can be found in the report for the parent project 5450-51000-039-00D, Mineral Intakes for Optimal Bone Development and Health

The purpose of this research is to determine whether silicon as a novel arginine silicate inositol complex or sodium metasilicate prevents any silicon deprivation-enhanced undesirable changes in markers of bone and connective tissue metabolism induced by pro-inflammatory agents. The experimental portion of the research has been completed. A peer-reviewed publication that is listed in the report for the parent project showed that physiological amounts of silicon promote the immune response; sex may influence the response to dietary silicon, and both organic silicon complexes (i.e., arginine silicate inositol complex) and inorganic silicon are similarly effective in preventing changes in inflammation induced by silicon deprivation. Another publication will be prepared indicating that injection of a substance that results in an inflammatory response (lipopolysaccharide, LPS, a cell wall component of Gram-negative bacteria) induces a redistribution of silicon in the body. This finding supports the suggestion that silicon is beneficial for wound healing and the immune

Project Number: 5450-51000-039-07T

Accession: 0408848

FY: 2008

response, and that silicon may affect bone health through influencing the inflammatory response.

Progress in this project was communicated to Nutrition 21, Inc. by the ADODR through telephone calls, emails, and personal contacts at scientific meetings.

4. Accomplishments:

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/23/2008

Project Number: 5450-51000-039-08T Accession: 0412602 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: JAY J CAO

Start Date: 10/01/2007 Term Date: 12/31/2008

National Programs: 107 N Human Nutrition

Title: CALCIUM RETENTION AS INFLUENCED BY BEEF AND OTHER DIETARY COMPONENTS THAT INDUCE AN
ACID LOAD IN POSTMENOPAUSAL WOMENPeriod Covered From: 10/2007 To: 9 / 2008 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-8-0404

Organization Name: NATIONAL CATTLEMEN'S BEEF ASSOCIATION

Progress and Outcomes:

1a. Objectives (from AD-416)

This investigation will test the following hypotheses in a 15-wk controlled diet study of healthy postmenopausal women:

1. Dietary calcium is retained as well from a diet high in meat protein and potential acid load as it is from a diet low in meat protein and potential acid load.
2. Urinary acid and calcium excretion adapt over time (within weeks) to diets with high- or low-acid loads.

1b. Approach (from AD-416)

General Approach - Twenty normal and healthy post-menopausal women will participate in the study. The volunteers will consume two diets, one low in protein (10% of energy), meat (20 g/d, 15 g/d as beef) and potential renal acid load (-17 mEq/d) and one high in protein (20% of energy), meat (74 g/d, 51 g/d as beef) and potential renal acid load (46 mEq/d). The diets will be consumed for 7 weeks each with a one week washout period (15 weeks total) in a randomized cross-over design. Both diets will contain approximately 700 mg calculated calcium to match the typical intakes of postmenopausal women. After 3 wk dietary equilibration (e.g., weeks 4 and 11), the entire 2-d menu of each diet will be radio-labeled with ^{47}Ca . Calcium retention will be measured by whole body scintillation counting for 4 weeks after ingestion of the radio-labeled meals. Blood and urine samples will be collected to assess the effects of the diet on biomarkers of bone metabolism and renal adaptation to the acid-load. To screen for normal bone health, the bone mineral density, the volunteers will be measured by dual x-ray absorptiometry.

3. Progress Report

This report serves to document research conducted under a Trust Fund Cooperative Agreement between ARS and the National Cattlemen's Beef Association. Additional details of research can be found in the report of the parent project 5450-51000-039-00D, Mineral Intakes for Optimal Bone Development and Health

To determine whether high-meat protein with high acid load influences calcium metabolism, 20 post-menopausal women consumed two experimental diets, high-meat protein (high acid load) and low-meat protein (low acid load) for seven (7) weeks each with one week break in a randomized crossover design. Calcium retention from two sets of two-day ^{47}Ca -labeled meals was measured by whole body scintillation counting. The dietary

Project Number: 5450-51000-039-08T

Accession: 0412602

FY: 2008

intervention is completed and chemical analyses are in process.

ADODR monitoring activities to evaluate research progress included electronic mail correspondence.

4. Accomplishments:

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/29/2008

Project Number: 5450-51530-009-00D Accession: 0408299 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: HENRY C LUKASKI

Start Date: 04/03/2004 Term Date: 04/02/2009

National Programs: 107 N Human Nutrition

Title: MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

Period Covered From: 10 / 2007 To: 9 / 2008 Final Report? No
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Improve health and enhance quality of life by determining, for healthy and at-risk populations (e.g., school-aged children, rural elderly, Native Americans), mineral intakes that promote optimal physiological and psychological development, function and health; develop new functional bases for establishing mineral element requirements; identify mechanisms of action; and determine the influence of sex, age, genetic, lifestyle and environmental factors on mineral element requirements. Develop and implement health promoting interventions for prevention of obesity and co-morbidities in American Indian population in the upper Midwest.

1b. Approach (from AD-416)

Dietary intakes and biochemical indices of mineral status will be related to physiologic (e.g., body composition, weight maintenance, physical fitness, energy metabolism, brain and cardiac function) and psychological (e.g., cognition, emotional and social adjustment, school/work performance) measures to determine roles of specific minerals in supporting optimal function and development. A Mobile Nutrition Research Laboratory, Community Studies Unit, and a residential Metabolic Research Unit will be used to conduct epidemiologic, supplementation, fortification, and controlled feeding studies, respectively with healthy and at-risk subjects (e.g., school-aged children, rural elderly, Native Americans). Use qualitative assessment methods (focus groups and in-depth interviews) and surveys to develop and implement social ecological, culturally-sensitive and scientifically sound interventions in American Indian communities. Randomized controlled trials will evaluate the effects of graded intakes of minerals, such as iron, zinc, copper, manganese and boron, and mediating factors (e.g., genotype, controlled stressors). Animal studies will be used to determine the mechanisms of action of functional outcomes. Studies will involve university, industry and government collaboration.

2. Milestones for FY2008

1. Plan preliminary feasibility study of physical activity components of ARS Obesity Prevention Initiative.
Milestone Substantially Met
2. Determine the barriers and facilitators affecting adherence to Dietary Guidelines for Americans in college students and American Indians at Spirit Lake reservation.
Milestone Fully Met
3. Plan survey of community/culturally-specific diets and physical activities that promote health among Native American Communities.

Project Number: 5450-51530-009-00D

Accession: 0408299

FY: 2008

Milestone Substantially Met

4. Analyze results of relationship between zinc and iron nutrition and cognitive function in children and adolescents.

Milestone Substantially Met

5. Analyze data on dietary copper and adaptation to exercise in rodents.

Milestone Substantially Met

6. Analyze samples and data from study of dietary zinc and adaptation to exercise in rodents.

Milestone Substantially Met

7. Plan and initiate study of dietary copper and exercise in high-capacity rats.

Milestone Not Met**Other**

Collaborator did not provide the out-bred rats because his NIH grant was not funded.

8. Conduct study of interaction of dietary boron and essential fatty acids on neurological function.

Milestone Substantially Met**3. Progress Report**

People who experience food insecurity are at increased risk of obesity and chronic diseases. In collaboration with a coalition of public and non-public organizations, scientists from the Center conducted a state-wide survey of the charitable feeding network in North Dakota. This project, which was funded by the Otto Bremer Foundation, gathered information from focus groups, a postcard survey completed by more than 1,800 households, and a questionnaire distributed to all existing emergency food providers; local, state and federal statistics also were used. It was determined that one in 12 North Dakotans get assistance from the state's network of charitable food providers. Information from this study will be used to develop programs to eliminate hunger in rural and urban communities throughout the state. (NP107, Component 6: Prevention of Obesity and Disease: Relationship between Diet, Genetics, and Lifestyle)

Zinc and copper are limiting nutrients in some segments of the population. As people increase participation in physical activities to enhance health, there is a need to understand the role of these minerals in regulating physiological adaptations to exercise. We conducted experiments with rodents that were fed diets with intakes of copper and zinc (1.0 and 5 mg/d, respectively) that elicit marginal, not severe, deficiencies similar to that observed in humans. The outcome measures included carbonic anhydrase (zinc-dependent) enzyme in skeletal muscles, cytochrome c oxidase (copper-containing) enzyme, peroxisome proliferator-activated receptor gamma (nuclear receptor protein regulating metabolic adaptations in cells), and histological changes in muscle fiber types and lipid accumulation. Some of this work utilized inbred strains of rats with different phenotypes for physical activity. (NP107, Component 4: Nutrient Requirements)

A previous experiment at Grand Forks, ND, indicated that behavioral characteristics and brain composition of boron-deficient, but not boron-adequate, rats fed safflower oil (high in omega-6 fatty acids) instead of fish oil (high in omega-3 fatty acids) were undesirably affected. Thus, an experiment was conducted with rats to confirm that diets high in omega-6 fatty acids exacerbate, or diets high in omega-3 fatty acids, attenuate brain composition and behavioral changes induced by boron deficiency. Behavioral responses of the rats to the dietary treatments have been completed and only

Project Number: 5450-51530-009-00D

Accession: 0408299

FY: 2008

one brain composition variable remains to be determined. Preliminary evaluation of the data obtained indicates that the fatty acid composition of the diet influences the effect of boron deprivation on the activity of experimental animals. (NP107, Component 4: Nutrient Requirements)

4. Accomplishments:

- 1 Low Dietary Copper Similar to an Amount Consumed by the US Public Impairs Health Benefits Induced by Physical Activity.

Regular physical activity induces beneficial changes in skeletal muscle structure and biochemical functions related to energy expenditure. Copper regulates the activity of cytochrome c oxidase, an enzyme that controls the production of the energy molecule, adenosine triphosphate, needed for muscles to contract. The consequences of restricted copper intake on muscle structure and function during physical activity such as moderate-intensity endurance training are unknown. We determined the effects of marginally deficient and adequate dietary copper on skeletal muscle composition and enzyme activities of female rats with different phenotypes for running capacity and assigned to either endurance training or no training. Regardless of running capacity, marginal copper hampered the transformation of muscle fibers to a fiber type conducive to aerobic exercise. The higher dietary copper promoted beneficial changes in skeletal muscle morphology only in the low-capacity rats compared to the untrained rats. Cytochrome c oxidase activity increased in response to higher dietary copper more in the low- compared to the high-capacity rats. These findings indicate that copper is needed for beneficial changes induced by physical activity but a marginal copper intake impairs these changes more in rats with a phenotype for low-capacity than a high-capacity for endurance training. IMPACT: Individuals who seek to engage in regular physical activity to gain health benefits should ensure an adequate intake of copper to manifest functional changes in skeletal muscle. (NP107, Component 6: Prevention of Obesity and Disease: Relationship between Diet, Genetics, and Lifestyle)

5. Significant Activities that Support Special Target Populations

Scientists in the unit continue to work with American Indians to develop successful partnerships and to promote research on health promotion. Examples include the continuation of a Cultural Awareness Workshop at United Tribes Community College, attended by the researchers, technicians and administrators from throughout the Northern Plains Area. These activities directly support Grand Forks Human Nutrition Research Center programs to improve the nutrition and health of this at-risk and underserved population in our region, and facilitate accomplishment of the milestone related to health promotion and obesity prevention in American Indian communities.

In addition, an important Specific Cooperative Agreement was completed to promote collaborative research partnerships with Cankdeska Cikana Community College (Spirit Lake Reservation). This agreement seeks to formalize relationships to initiate discussion geared to develop culturally-appropriate activities and intervention to promote health and prevent obesity and diabetes among American Indians in the Northern Great Plains. One project completed during this past year used qualitative methods to identify and characterize barriers and facilitators associated with potential nutrition and lifestyle changes to achieve and maintain a healthy body weight and reduce risk of chronic diseases and mitigate disease symptoms at Cankdeska Cikana Community College (Spirit Lake Reservation). A second health needs survey to assess prevalence of asthma at the Spirit Lake Dakota Nation was recently completed. This information will be used to develop research project to assess nutritional intakes, and to determine whether differences exist in the intakes of

Project Number: 5450-51530-009-00D

Accession: 0408299

FY: 2008

anti-oxidant and other bioactive components of food between of children and adolescents with and without medically-defined asthma. This work was accomplished in the subordinate project (#5450-51530-009-05S).

6. Technology Transfer

- 0 Number of New CRADAs
- 0 Number of Active CRADAs
- 0 Number of New MTAs (providing only)
- 0 Number of Invention Disclosures Submitted
- 0 Number of Patent Applications Filed
- 0 Number of New Germplasm Releases
- 0 Number of new commercial licenses granted
- 0 Number of web sites managed
- 5 Number of non-peer reviewed presentations and proceedings
- 2 Number of newspaper articles and other presentations for non-science audiences
- 1 Number of Other Technology

Non Peer Reviewed Presentations and Proceedings Details:

- 01 **Description:** Technology: Strategic Plan for Spirit Lake Tribe 2008-2013.
Transfer: Written report of the priority areas and actions to accomplish the strategic plan for the Spirit Lake Tribe. (September 2007).
Customer/User: Tribal government leaders, public health officials, wellness promoters, and nutrition professionals.
Impact/Outcome: A formal plan identifying the priorities of the Spirit Lake Tribe to improve the health and well-being of the tribal members and developing an action plan for use by local governing agencies.
- 02 **Description:** Technology: Compilation of estimates of mineral losses in sweat during exercise in hot environments.
Transfer: Oral presentation was given at the VIIIth Conference of the International Society for Trace Element Research in Humans (Lukaski, H.C. Mineral Losses during Extreme Environmental Conditions, Hersonissos, Crete, Greece, October 24, 2007).
Customer/User: Other scientists, food and beverage industry representatives, nutrition professionals.
Impact/Outcome: Revised estimates of trace element losses during physical activity needed to update recommendations for dietary mineral intakes.
- 03 **Description:** Technology: Creating a Hunger-Free North Dakota.
Transfer: Written report prepared by public and non-profit organizations of the status of charitable feeding network in North Dakota including barriers to access and the need to expand these services.
Customer/User: Community-action programs and local food assistance programs.
Impact/Outcome: Implementation of new initiatives to improve service to rural and under-served communities by increased coordination with national retailers in the state and strengthening of existing food-assistance organizations by building capacity to serve more people.

Project Number: 5450-51530-009-00D

Accession: 0408299

FY: 2008

- 04 **Description:** Technology: Vitamin and mineral needs for physical performance.
Transfer: Tele-seminar, web-access broadcast, July 2008.
• www.TheFitnessPhone.com, July 2008.
Customer/User: Physically-active people, dietitians, coaches, other scientists.
Impact/Outcome: Improve eating habits to optimize vitamin and mineral intakes to meet the needs of physically-active children, adolescents and adults.
- 05 **Description:** Technology: Adequate dietary magnesium prevents inflammation and oxidative stress that increase the risk for chronic disease.
Transfer: Poster and oral presentations (Nielsen, F.H. Response of Rats to 50% of the Estimated Dietary Magnesium Requirement Changes with Length of Deprivation and Different Dietary Fat Sources) at the Gordon Conference: Magnesium in Biochemical Processes & Medicine, Ventura, CA, March 2008.
Customer/User: Other scientists, nutrition professionals, food and beverage industry representatives.
Impact/Outcome: Reduce inflammation, oxidative stress, and circulating homocysteine that increase the risk for cardiovascular disease in people by encouraging the consumption of diets high in magnesium-rich foods.

Newspaper Articles and Other Presentations for Non Science Audiences Details:

- 01 **Description:** Technology: Consumption of diets high in whole-grains induces genes to make proteins that decrease risk factors for ischemic heart disease in humans.
Transfer: Newspaper
• Grand Forks Herald, September 2007
Customer/User: General public, nutrition and health professionals, other scientists
Impact/Outcome: Reduce cholesterol and low-density lipoproteins in blood of people.
- 02 **Description:** Technology: Dietary fatty acids and magnesium decrease inflammation and reduce risk for chronic disease.
Transfer: Newspaper
• Grand Forks Herald, June 2008
Customer/User: General public, nutrition and health professionals, other scientists
Impact/Outcome: Reduce inflammation that increases the risk for chronic diseases such as atherosclerosis and osteoporosis in people by encouraging the consumption of diets high in foods rich in n-3 fatty acids and magnesium.

Other Technology Details:

- 01 **Description:** Technology: Nutrient composition of traditional Native American plant foods
Transfer: USDA National Nutrient Database for Standard Reference, Release 20, February 2008.
Customer/User: Indian Health Service, dietetic and nutritional professionals, other scientists
Impact/Outcome: This is the only nutrient database for plant foods consumed by Northern Plains Native Americans, and it enables planning

Project Number: 5450-51530-009-00D

Accession: 0408299

FY: 2008

of health-promoting diets using culturally-appropriate foods.

7. International Cooperation / Collaboration

01 KENYA

We are collaborating with investigators from the US Army Research Institute of Environmental Medicine (USARIEM) and the US Army Medical Research Unit - Kenya and the Kenya Medical Research Institute/Walter Reed Project in a double-blind supplementation trial to test the hypothesis that zinc supplementation reduces the incidence and symptoms of diarrhea, and decreases the presence of bacterial vectors associated with diarrhea. This project is under cooperative agreement 5450-51530-009-10R.

Scientific Publications:

Log 115

1. Chinevere, T.D., Kenefick, R.W., Cheuvront, S.N., Lukaski, H.C., Sawka, M.N. 2008. Effect of Heat Acclimation on Sweat Minerals. *Medicine and Science in Sports and Exercise*. 40(5):886-891 000022048
2. Droke, E.A, Lukaski, H.C. 2008. Dietary fatty acids and minerals. In: Chow, C.K., editor. *Fatty Acids In Foods and their Health Implications*. 3rd edition. Boca Raton, FL: CRC Press. p 631-650. 000020172
3. Lam, P.K., Kritz-Silverstein, D., Barrett-Connor, E., Nielsen, F.H., Milne, D., Gamst, A., Morton, D., Wingard, D. 2008. Plasma trace elements and cognitive function in older men and women: The Rancho Bernardo Study. *Journal of Nutrition Health and Aging*. 12(1):22-27. 000017211
4. Montain, S.J., Cheuvront, S.N., Lukaski, H.C. 2007. Sweat mineral element responses during 7 h of exercise-heat stress. *International Journal of Sport Nutrition*. 17(6):574-582. 000020997
5. Nielsen, F.H. 2007. Trace mineral deficiencies. In: Berdanier, C.D., Dwyer, J., Feldman, E.B., editors. *Handbook of Nutrition and Food*. Second Edition. Boca Raton, FL. CRC Press. p.159-176. 000019313
6. Shafer, K.J., Siders, W.A., Johnson, L., Lukaski, H.C. 2008. Interaction of clothing and body mass index affects validity of air displacement plethysmography in adults. *Nutrition*. 24(2):148-154. 000020645
7. Yokoi, K., Egger, N.G., Ramanujam, V.M., Alcock, N.W., Dayal, H.H., Penland, J.G., Sandstead, H.H. 2007. Association between zinc pool sizes and iron stores in premenopausal women. *British Journal of Nutrition*. 98(6):1214-23. 000019031

Approved: MCGUIRE MICHAEL R

Date: 09/17/2008

Project Number: 5450-51530-009-03N Accession: 0409328 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: HENRY C LUKASKI

Start Date: 05/26/2005 Term Date: 03/31/2009

National Programs: 107 N Human Nutrition

Title: ASSESSMENT OF MINERAL LOSSES IN SWEAT DURING PHYSICAL ACTIVITY

Period Covered From: 10 / 2007 To: 9 / 2008 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-5-0107N

Organization Name: GATORADE SPORTS SCIENCE INSTIT.

Progress and Outcomes:

1a. Objectives (from AD-416)

This project will evaluate the feasibility of determining sweat losses of mineral elements in humans during periods of controlled physical activity. This study is an initial effort to develop a valid and reliable method to determine surface mineral losses. This method is needed to improve assessment of mineral nutritional needs of physically active people.

1b. Approach (from AD-416)

The cooperator will recruit and enroll skilled athletes to participate in the approved study to determine the effects of physical activity on body sweat losses. The cooperator will provide unique sweat collection apparatus and position them on various sites of the body before controlled physical activities, monitor mineral-containing beverage intake, then remove the sweat collection devices. Sweat will be extracted and sent to the USDA, ARS Grand Forks Human Nutrition Research Center for determination of mineral concentrations. Data will be shared between ARS and cooperator scientists and prepared for publication in a scientific journal.

3. Progress Report

This report serves to document research conducted under a Non-Funded Cooperative Agreement between ARS and the Gatorade Sport Science Institute. Additional information can be found in the report for the parent project 5450-51530-009-00D Micronutrient Roles in Physiology and Health

Progress includes the development and construction of an open chamber for the collection of whole-body sweat during exercise of humans in various environmental conditions of heat and humidity. Preliminary experiments demonstrated that the concentrations of sodium, potassium, calcium, copper, iron, magnesium, manganese, and zinc in distilled-deionized water applied to the surfaces of the chamber were zero. This finding establishes that there is no external contamination caused by the materials in contact with sweat. A second experiment found 93-98% recovery of a mineral and trace element-defined, water-based solution similar in composition to human sweat and in contact with the interior of the chamber. These data further demonstrate the lack of appreciable contamination from the materials in the sweat-collection chamber. Results of these two experiments establish that the testing/collection chamber is not a significant source of electrolyte and mineral contamination. Human studies are being planned to determine the electrolyte and trace element losses of adults and children during exercise in hot and humid conditions and some dietary

Project Number: 5450-51530-009-03N

Accession: 0409328

FY: 2008

interventions.

Activities were monitored by using monthly conference calls and discussions at scientific conferences.

4. Accomplishments:

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/23/2008

Project Number: 5450-51530-009-04N Accession: 0410137 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: GERALD F COMBS

Start Date: 03/09/2006 Term Date: 09/30/2010

National Programs: 107 N Human Nutrition

Title: MINERAL NUTRITION RESEARCH

Period Covered From: 10/2007 To: 9 /2008 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-6-0101N

Organization Name: US ARMY RES INST ENVIR MEDICINE

Progress and Outcomes:

1a. Objectives (from AD-416)

Collaborator in planning, implementation and reporting of research on the effects of minerals on human nutritional needs and physical and psychological performance.

1b. Approach (from AD-416)

Human volunteers will be studied under a variety of dietary conditions and biochemical and functional parameters will be measured.

3. Progress Report

This report serves to document research conducted under a non-funded cooperative agreement between ARS and the United States Army Research Institute for Environmental Medicine (USARIEM). Additional details of research can be found in the report for the parent project, 5450-51530-009-00D, Micronutrient Roles in Physiology and Health

The project represents the commitment of the Grand Forks Human Nutrition Research Center and the USARIEM to collaborate in studies of mutual value in the general area of mineral nutrition. The focus of efforts this year was to modify existing analytical methods and to determine zinc concentrations in interstitial fluid. We tested the hypothesis that zinc concentrations in interstitial (fluid bathing cells) fluid were directly related to plasma zinc concentrations. We selected zinc as the variable of interest because it is a limiting nutrient in the diets of soldiers, and has been related to measures of physical performance. We found that interstitial zinc concentration was almost 10 times greater than plasma zinc concentration, and these measures were very highly correlated in soldiers who completed a rigorous test of high-intensity exercise in a hot environment. However, two men, who did not complete the exercise in the heat test because they had very high body temperatures, had interstitial zinc concentrations that were not related to their plasma zinc levels. This finding indicates impairment in maintaining plasma zinc concentrations and a predisposition of heat intolerance.

ADODR monitoring activities: Investigators participated in weekly conference calls, discussed experimental plans, reviewed data, and met at a national scientific conference to discuss findings, which were presented at a national conference. Two manuscripts describing collaborative work completed during this performance period are listed in the report for the parent project (5450-51530-009-00D).

4. Accomplishments:

Project Number: 5450-51530-009-04N

Accession: 0410137

FY: 2008

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 08/15/2008

Project Number: 5450-51530-009-05S Accession: 0411236 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: GERALD F COMBS

Start Date: 09/19/2006 Term Date: 08/31/2011

National Programs: 107 N Human Nutrition

Title: HEALTH PROMOTION IN AMERICAN INDIAN COMMUNITIES

Period Covered From: 10/2007 To: 9 /2008 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-6-0351

Organization Name: CANKDESKA CIKANA COMM COLLEGE

Progress and Outcomes:

1a. Objectives (from AD-416)

The broad objective of this cooperative research is to develop information useful in promoting health through improved nutrition and lifestyles. The specific objectives are to:

1. Develop understanding of the relationships of diet, lifestyle, and the prevalence of chronic diseases, particularly obesity, diabetes, and cardiovascular disease in American Indian peoples;
2. Identify health needs, and the barriers to and facilitators of meeting those needs in American Indian communities;
3. Determine the efficacy of community-based, health-promoting, intervention strategies in American Indian communities; and,
4. Increase research cooperation between American Indian colleges and USDA-ARS.

1b. Approach (from AD-416)

Identification and characterization of barriers to and facilitators of eating healthy diets and engaging in healthy lifestyles will be accomplished through a series of focus groups in American Indian communities. Focus groups will be designed to also identify and prioritize community needs as potential mediating factors. Subsequently, health-promoting intervention strategies will be developed and evaluated based on identified barriers and facilitators in the context of community needs and priorities. A research involving human subjects will be conducted with appropriate review and approval by the respective institutional review boards used by Cankdeska Cikana Community College and ARS.

3. Progress Report

This report serves to document research conducted under a Specific Cooperative Agreement between ARS and the Cankdeska Cikana Community College. Additional details of research can be found in the report for the parent project, 5450-51530-009-00D, Micronutrient Roles in Physiology and Health

This project represents the commitment of the Grand Forks Human Nutrition Research Center and the Cankdeska Cikana Community College to collaborate in studies of mutual value to promote the health of American Indians. Based on limited information, the prevalence of asthma at the Spirit Lake Dakota Nation exceeds 20%, which is twice the reported rate in inner city and other Native American communities. To better define the prevalence of asthma among the youth, who make up more than 50% of the Spirit Lake Dakota Nation, a culturally-sensitive questionnaire was developed and included in a

Project Number: 5450-51530-009-05S

Accession: 0411236

FY: 2008

needs assessment survey conducted in each household. Results will be available later this year. It is hypothesized that a nutritional factor, either an imbalance in the ratio of n-6 to n-3 fatty acids or lack of food-derived antioxidants, beyond traditional environmental factors (second hand smoke, pet dander and mold) may be causative. After the results of the asthma survey are available, we plan to engage the tribal community in an assessment of diet composition and a comparison of nutrient intakes of age- and gender-matched children with and without asthma.

Activities were monitored by frequent site visits at Cankdeska Cikana Community College and regular conference calls including discussions for planning activities among the investigators.

4. Accomplishments:

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/25/2008

Project Number: 5450-51530-009-07N Accession: 0411613 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: HENRY C LUKASKI

Start Date: 02/01/2007 Term Date: 04/02/2009

National Programs: 107 N Human Nutrition

Title: DIET AND EXERCISE ON PROTEIN EXPRESSION IN MUSCLE

Period Covered From: 10/2007 To: 9 /2008 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-7-0110N

Organization Name: US ARMY RES INST ENVIR MEDICINE

Progress and Outcomes:

1a. Objectives (from AD-416)

To identify micro-nutrient responsive proteins in muscle and other tissues obtained from rodent models.

1b. Approach (from AD-416)

Laboratory rodents of varying ages and, in some cases varying genotypes and phenotypes, will be fed diets containing micronutrients in marginally-deficient and adequate amounts and either exposed to physical training or untrained. Comparisons will be made among established biochemical and physical markers of nutritional status and expression of proteins in selected tissues to determine impacts of sub-clinical micronutrient deficiencies. Emphasis will be muscle mitochondrial complexes and signal transduction in mitogenesis and angiogenesis.

3. Progress Report

This report serves to document research conducted under a Non-Funded Cooperative Agreement between ARS and the United States Army Research Institute for Environmental Medicine (USARIEM). Additional details of research can be found in the report for the parent project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health

The project represents the commitment of the Grand Forks Human Nutrition Research Center and the USARIEM to collaborate in studies of mutual value in the broad area of interaction of diet and physical activity on expression of proteins that regulate structural and functional adaptations of skeletal muscle and other key organs. One study was completed; it examined the effect of different durations of swimming on the expression of a unique protein found in the nucleus of cells that controls the ability of muscles to use fat and carbohydrate to produce energy. We found that two hours of swimming daily for 2-3 weeks modestly elevated the amount of this protein whereas a six hour bout of swimming for five days elicited a much greater increase in the amount of this vital protein. Because prolonged, strenuous exercise can reduce food intake, we hypothesized that voluntary food (energy) restriction associated with the longer exercise period would secondarily increase the protein production. However, because we used a non-exercised, food-matched group of rats, we found no change in the amount of this protein with modest decreases in food intake. These findings show for the first time that prolonged exercise, and not exercise-induced food (energy) restriction, was responsible for increasing levels of this protein in skeletal muscle.

Investigators and staff participated in frequent conference calls to discuss

Project Number: 5450-51530-009-07N

Accession: 0411613

FY: 2008

experimental plans and progress in conducting the experiments, and to review results and preliminary findings.

4. Accomplishments:**7. International Cooperation / Collaboration**

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/23/2008

Project Number: 5450-51530-009-08T Accession: 0412129 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: FORREST H NIELSEN

Start Date: 05/01/2007 Term Date: 05/01/2009

National Programs: 107 N Human Nutrition

Title: MAGNESIUM NUTRITION AND SLEEP BEHAVIOR IN OLDER ADULTS

Period Covered From: 10 / 2007 To: 9 / 2008 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-7-0431

Organization Name: TYRASE RESEARCH

Progress and Outcomes:

1a. Objectives (from AD-416)

The objective of this cooperative research project is to determine the association between magnesium nutrition (intakes and status) and sleep behavior (quantity, quality, disturbances) in older adults; to determine the efficacy of magnesium supplementation to improve sleep (increase quantity and quality and prevent or reduce disorders); and to identify factors (for example, gender, health, diet, body composition, physical activity, depression - historical or current) that mediate or moderate the relationship between magnesium nutrition and sleep.

1b. Approach (from AD-416)

An experiment will be performed that will have an 8-week double-blind placebo-controlled cross-sectional design. People with sleep complaints (for example, insomnia, nighttime awakenings, difficulty in falling asleep, awakening too early, not feeling rested after sleep) will be recruited. Following baseline assessment during week one of past and current health, diet, body composition, physical activity, depression, and sleep, 100 adults (50 males and 50 females) aged older than 51 yrs will be randomly assigned to one of two groups of 50 each. Groups will be matched by gender, age and overall sleep score and magnesium status (determined by erythrocyte magnesium and calcium, and plasma total and ionized magnesium). Then one group will be given a 300 mg/day magnesium gluconate supplement for 7 weeks while the other group will be given a placebo. An assessment of health, diet, body composition, physical activity, depression, sleep and magnesium status will occur during weeks 6 and 8, concluding the study.

3. Progress Report

This report serves to document research conducted under a Trust Fund Cooperative Agreement between ARS and Tyrase Research, Grand Forks, ND. Additional details of research can be found in the report of the parent project 5450-51530-009-00D, Micronutrients Roles in Physiology and Health

The purpose of this research is to determine whether a low magnesium status contributes to the Whigh prevalence of sleep disturbances in older adults. Final approvals to do the study and recruitment of volunteers began in November 2007. Information meetings were held January 15 & 24, February 13 & 20, March 19 & 27, April 16 & 24, May 28, and July 1, 2008. At the end of July 2008, 415 people expressed interest in the study, 220 were acceptable for on-site interviews, 118 completed lab work to determine final eligibility, and 92 were enrolled in the study. After enrollment, 76 completed the

Project Number: 5450-51530-009-08T

Accession: 0412129

FY: 2008

study, 6 are currently participating or will participate in the study, 3 subjects were discharged and 7 subjects withdrew. Evaluation of the data obtained will be done after all subjects have completed the study.

Because the study is being performed in-house, the ADODR is actively participating in the research by conducting information meetings, assessing the validity of the procedures followed, and addressing problems with equipment, forms and procedures occurring during the study. Progress in this project was communicated to Tyrase Research by the ADODR through telephone calls.

4. Accomplishments:

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/23/2008

Project Number: 5450-51530-009-10R Accession: 0412541 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
 GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
 NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH

Prin Invs: HENRY C LUKASKI

Start Date: 10/01/2007

Term Date: 09/30/2008

National Programs: 107 N Human Nutrition

Title: EFFICACY OF ZINC SUPPLEMENTATION ON DIARRHEA INCIDENCE IN AN ADULT POPULATION IN
WESTERN KENYA

Period Covered From: 10 / 2007 To: 9 / 2008

Final Report? No

Terminate in Two Months? No

Agreement Number: 60-5450-8-0400

Organization Name: U.S. ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE

Progress and Outcomes:

1a. Objectives (from AD-416)

ARS will receive blood samples and measure blood biochemical indicators of iron, zinc, selenium nutritional status and indicators of inflammation of human volunteers.

ARS will ship samples for vitamin A and retinol-binding protein to Pennington Research Center where USARIEM has a contract to run their blood chemistries.

ARS will provide the results of analytical tests to US Army Research Institute of Environmental Medicine (USARIEM) co-investigators.

USARIEM will recruit human volunteers, randomize volunteers to treatment groups, and provide supplements and placebo treatments.

USARIEM will obtain, prepare and ship blood samples to ARS.

USARIEM will recruit and train field research associates who will collect data on diarrhea incidence and symptoms.

USARIEM collaborators will evaluate the effects of zinc supplementation on clinical assessments of diarrhea and some potential mediating factors.

ARS and USARIEM jointly will participate in interpretation of findings and preparation of reports and manuscripts.

1b. Approach (from AD-416)

The study will use a double-blind (observer blind and volunteer blind), randomized controlled design (randomization ratio 1:1) of supplemental zinc (20 mg/d) compared to placebo (maltodextrin) for five months. Randomization will include a total of 500 eligible adults matched by sex and age (18 to 55 years) and living in Western Kenya. This field research will be supervised and coordinated by investigators of the US Army Medical Research Unit - Kenya (USAMRU-K) and the Kenya Medical Research Institute (KEMRI)/Walter Reed Project (WRP), and conducted at the Kombewa Clinical Research Center (KCRC) outside of Kisumu in Western Kenya. This study will test the hypothesis that zinc supplementation reduces the incidence and symptoms of diarrhea and decreases presence of bacterial vectors associated with diarrhea.

3. Progress Report

This report serves to document research conducted under a Reimbursable Cooperative Agreement between ARS and the United States Army Research Institute for Environmental Medicine (USARIEM). Additional details of research can be found in the report for the parent project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health

Project Number: 5450-51530-009-10R

Accession: 0412541

FY: 2008

The project represents the commitment of the Grand Forks Human Nutrition Research Center and the USARIEM to collaborate in studies of mutual value in the broad area of nutritional supplementation in the prevention of diarrhea in an area with chronic diarrheal infection. During FY07, we completed the zinc supplementation trial. All blood samples were analyzed for C-reactive protein, serum ferritin, and plasma concentrations of trace elements and electrolytes. Data analysis is underway to determine the effect of supplemental zinc on incidence of diarrhea and pathogens responsible for diarrhea in Kenyan adults.

Investigators and staff participated in frequent conference calls to discuss experimental plans, progress in conducting the trial, transportation of samples from Kenya to the GFHNRC, and to review analytical results and preliminary findings.

4. Accomplishments:

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/29/2008

Project Number: 5450-51530-009-11N Accession: 0412737 FY: 2008

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Prin Invs: GERALD F COMBS

Start Date: 01/01/2008 Term Date: 12/31/2012

National Programs: 107 N Human Nutrition

Title: GRAND FORKS COMMUNITY-BASED HEALTH AND FITNESS AGENDA

Period Covered From: 10 / 2007 To: 9 / 2008 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-8-0101N

Organization Name: GRAND FORKS PARK DISTRICT

Progress and Outcomes:

1a. Objectives (from AD-416)

To develop a long-term partnership to foster the development of effective wellness/fitness programs in the Greater Grand Forks Community that will provide opportunities for community based research addressing issues related to the prevention of obesity.

1b. Approach (from AD-416)

The Grand Forks Parks District (GFPD) will work with community groups to develop wellness/fitness programs and facilities in the Greater Grand Forks Community. The Grand Forks Human Nutrition Research Center (GFHNRC) will work with the GFPD to advise on issues related to the health needs of citizens, and the design and implementation of those programs/facilities. Both institutions will work together to identify strategic linkages that will meet the goals of the GFPD and advance the research mission of the GFHNRC.

3. Progress Report

This report serves to document research conducted under a Non-Funded Cooperative Agreement between ARS and the Grand Forks Park District. Additional details of research can be found in the report for the parent project 5450-51000-009-00D, MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

This collaboration planned and implemented a pilot program of healthy food choices and physical activity for 311 school-aged children enrolled in Park District summer programs. This used the "eat the rainbow" approach and linked the concepts of low-fat, low-calorie snack and physical activity choices to health and fun. The purpose of the pilot was to gauge community interest in health-related programming and to determine the suitability and outfitting needs of the GFHNRC mobile nutrition laboratory for such use. The experience proved useful in this regard, enabling us to prepare appropriately for conducting obesity prevention research (surveys and interventions) nested in the context of such a summer program. The collaboration also arranged a series of community meetings addressing obesity prevention and healthy food choices. ADODR monitoring included phone calls, meetings, and conference calls.

4. Accomplishments:

Project Number: 5450-51530-009-11N

Accession: 0412737

FY: 2008

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/23/2008

MICRONUTRIENT ABSORPTION AND METABOLISM

MANAGEMENT UNIT

5450-020-00

Project Number: 5450-51000-035-00D Accession: 0407991 FY: 2008

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Prin Invs: JANET ROSS HUNT

Start Date: 01/15/2004

Term Date: 01/14/2009

National Programs: 107 N Human Nutrition

Title: MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES

Period Covered From: 10/2007 To: 9 / 2008

Final Report? No

Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

The general objective is to determine how current and proposed changes to the American diet that may adversely affect intake and/or how bioavailability of the essential mineral nutrients can be modified to enhance trace element nutrition, with emphasis on selenium (Se), iron (Fe), zinc (Zn), and copper (Cu). Specific objectives are:

Objective 1: Determine how shifts in agricultural and dietary practices, such as the availability of functional/genetically modified foods and emphasis on plant-based diets with reductions in meat consumption will impact the intake, bioavailability, and dietary requirements of minerals. This objective will address the production of foods with enhanced bioactive Se compounds, and assess their ability to enhance health, especially by controlling oxidative stress and reducing cancer risk. The impact of organic farming methods will also be assessed (Finley). It will also address the practical impact of dietary changes that emphasize plant-based diets on meeting nutritional needs for Fe and Zn (Hunt).

Objective 2: Determine the effectiveness of current and proposed mineral fortification/supplementation practices for improving mineral nutrition while avoiding excessive or imbalanced mineral intakes. This objective will evaluate the bioavailability of Fe fortificants such as elemental Fe and micronized, encapsulated Fe compounds in human studies (Hunt).

Objective 3: Determine the mechanisms of uptake, transport, and retention of food minerals and how mineral nutritional status influences these mechanisms to impact the bioavailability of essential minerals, non-nutritive metals, and other food components. Cell and whole animal models will be employed to elucidate how the modifications of mineral content of foods can influence the biochemical regulation of specific transporters, cellular trafficking, and interactions of minerals such as Zn, Fe, Cu, Cd, Se, and Mn. (Reeves).

Problem to be addressed with increased funds: Elucidate the roles and diets in support of optimal health and prevention of obesity and related illnesses, cardiovascular disease, osteoporosis and cancer.

Problem to be addressed with increased funds (FY05): Under Performance Measure 4.1.1 of the ARS Strategic Plan and the NP107 Action Plan, this project will develop an enhancement to the food supply by increasing the nutritional value of beef.

Project Number: 5450-51000-035-00D

Accession: 0407991

FY: 2008

Objective modification FY05: Increase the amount of omega-3 fatty acids in beef to a nutritionally significant level by feeding flax. Demonstrate that the increase in omega-3 fatty acids in the meat are sufficient to have a physiological effect. Study feasibility of increasing selenium in beef to levels that will have an impact on human health when the meat is consumed at recommended levels. This may include studies of organic form of selenium in beef, stability with varying cooking methods, sensory issues, bioavailability and health effects in both steers and consumers.

1b. Approach (from AD-416)

Methodology will include tests of agricultural conditions affecting the amounts and forms of minerals incorporated into in foods; in vitro, cellular, and animal models of mineral transport and absorption; and human experiments with controlled diets to assess mineral absorption, retention, and biological function and to model nutritional requirements.

Specific objectives to be accomplished with increased funding: To study the roles of foods, particularly those produced in the Northern Plains, in the support of health. This work is to be multi-disciplinary, including collaborations such as with the University of North Dakota School of Medicine and Health Sciences and North Dakota State University.

2. Milestones for FY2008

1. Obtain newly harvested high-Se soybeans
Milestone Fully Met
2. Complete Se analysis of high-Se soybeans
Milestone Fully Met
3. Complete protein isolation from high-Se soybeans
Milestone Fully Met
4. Complete study of phytate X Ca & Zn bioavailability
Milestone Fully Met
5. Report Zn requirement study
Milestone Fully Met
6. Complete study on validation of Caco-2 cell results with human absorption results on iron bioavailability from agricultural products
Milestone Fully Met
7. Plan and implement study to assess nonheme iron bioavailability from menus meeting the new Dietary Reference Intakes and Dietary Guidelines for Americans
Milestone Fully Met
8. Wrap up Cu/Heph/Fe absorption studies. Write manuscripts
Milestone Not Met
Critical vacancy (quantitative or qualitative deficiency in personnel)

3. Progress Report

To initiate research on the potential health benefits of high-Se soybeans, 2007-grown high-selenium soybeans were obtained from (1) the University Nebraska-Lincoln (experimentally produced) and (2) South Dakota producers (naturally occurring). High-

Project Number: 5450-51000-035-00D

Accession: 0407991

FY: 2008

Se yellow peas and oats were also obtained from South Dakota producers. Selenium analyses were completed, and the soybeans were processed to make isolated protein and tofu. Animal studies were begun to determine the bioavailability of selenium from the soy protein isolates (NE and SD), tofu (NE and SD), yellow peas and oats. (NP107, Component 2: Bioavailability of Nutrients and Food Components.)

To determine the impact of current dietary guideline recommendations on iron absorption, women's nonheme iron absorption was measured from menus planned to meet the dietary guidelines (MyPyramid 7-d sample menus). The study was successfully completed, and the data are being applied to help validate and further develop algorithms that predict iron absorption from dietary composition. (NP107, Component 2: Bioavailability of Nutrients and Food Components.)

Analysis and reporting of research on adaptation in human zinc absorption, as affected by zinc intake and bioavailability (especially dietary phytate) was completed, including statistical modeling of equations to predict zinc absorption from diets (see accomplishment below). (NP107, Component 2: Bioavailability of Nutrients and Food Components and Component 4: Nutrient Requirements.)

4. Accomplishments:

- 1 High bioavailability diets enable human to adaptively increase zinc absorption in response to low zinc intakes: Zinc is more efficiently absorbed from low zinc diets, but it is not known whether this reflects only a dose-response, or if human absorptive adapts to compensate for low zinc intakes, and how this may be affected by dietary bioavailability, especially phytic acid content. Healthy adults (n=109) participated in three human experiments to determine zinc absorption initially and after 4-8 wks consuming diets with a range of zinc contents, and with low or high phytic acid. The results showed that the initial greater efficiency of absorption from low zinc diets was further adaptively increased after several weeks consuming diets with less than 1 mg zinc per day, but only if the diets were low in phytic acid. Data modeling validated and improved a recently proposed formula to predict zinc absorption from dietary zinc and phytic acid. These results are useful for setting dietary zinc recommendations, which are based in part on estimates of dietary zinc absorption, and for improving human diets to reduce zinc deficiency globally. (NP107, Component 2: Bioavailability of Nutrients and Food Components and Component 4: Nutrient Requirements.)
- 2 Dietary guidelines emphasizing whole grains and other sources of phytic acid reduce iron absorption: Women's iron recommendations of 18 mg/d assume 18% average dietary iron absorption from US and Canadian diets by women with low body iron stores. However, food recommendations to consume more whole grains and legumes result in greater ingested phytate, an inhibitor of iron absorption. Adult women (n=13) absorbed 9% nonheme iron and 11% total iron from menus designed to meet current dietary recommendations. This was approximately one-third less efficient than that assumed in deriving the iron recommendations. These results suggest a greater risk of dietary iron deficiency with the food recommendations, and emphasize the need to further modify and balance nutrient and food guidelines to meet multiple nutritional goals. (NP107, Component 2: Bioavailability of Nutrients and Food Components and Component 4: Nutrient Requirements.)

5. Significant Activities that Support Special Target Populations

Project Number: 5450-51000-035-00D

Accession: 0407991

FY: 2008

None

6. Technology Transfer

- 0 Number of New CRADAs
- 0 Number of Active CRADAs
- 0 Number of New MTAs (providing only)
- 0 Number of Invention Disclosures Submitted
- 0 Number of Patent Applications Filed
- 0 Number of New Germplasm Releases
- 0 Number of new commercial licenses granted
- 0 Number of web sites managed
- 2 Number of non-peer reviewed presentations and proceedings
- 0 Number of newspaper articles and other presentations for non-science audiences
- 0 Number of Other Technology

Non Peer Reviewed Presentations and Proceedings Details:

- 01 **Description:** Technology: Hepcidin control of human iron absorption and metabolism
Transfer: Invited oral presentation given at the Experimental Biology meeting ("Prohepcidin and iron absorption in healthy women" EB2008 Symposium: Hepcidin Regulation of Iron Transport, San Diego, CA, April 6, 2008.)
Customer/User: Other scientists, health professionals.
Impact/outcome: The session was attended by ~200, and was videotaped for internet access, expanding opportunities for further research in developing a potentially valuable new indicator to distinguish between the anemia of iron deficiency and the anemia of inflammation.
- 02 **Description:** Technology: Improving bioavailable iron in staple food crops
Transfer: Invited oral presentation, "Comparison of relative bioavailability of iron from beans using Caco-2 cell model and human radioisotope techniques", at the Joint Technical Meeting of the International Atomic Energy Agency and HarvestPlus titled "Biofortification to improve micronutrient nutrition", IAEA Headquarters, Vienna, Austria; August 12-14, 2008.
Customer/User: Scientists in nutrition and crop breeding, to benefit consumers in developing countries.
Impact/outcome: Expanded opportunities for development of biofortified food crops.

7. International Cooperation / Collaboration**Scientific Publications:**

Log 115

- 1. Finley, J.W., Burrell, J., Reeves, P.G. 2007. Pinto Bean Consumption Changes SCFA Profiles in Fecal Fermentations, Bacterial Populations of the Lower Bowel, and Lipid Profiles in Blood of Humans. Journal of Nutrition. 137:2391-2398. 000021108
- 2. Hunt, J.R., Beiseigel, J.M., Johnson, L.K. 2008. Adaptation in human zinc absorption as influenced by dietary zinc and bioavailability. American Journal 000021683

Project Number: 5450-51000-035-00D

Accession: 0407991

FY: 2008

of Clinical Nutrition. 87(5):1336-1345.

3. Reeves, P.G., Chaney, R.L. 2008. Bioavailability as an issue in risk assessment and management of food cadmium: A review. Science of the Total Environment. 398(1-3):13-19.

000021234

Approved: MCGUIRE MICHAEL R

Date: 09/17/2008

Project Number: 5450-51000-035-18S Accession: 0407722 FY: 2008

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Prin Invs: JANET ROSS HUNT

Start Date: 09/29/2003

Term Date: 09/28/2008

National Programs: 107 N Human Nutrition

Title: HUMAN STUDIES RESEARCH

Period Covered From: 10/2007 To: 9 / 2008

Final Report? No

Terminate in Two Months? No

Agreement Number: 58-5450-3-0324

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

1a. Objectives (from AD-416)

To investigate the role of nutrients in human health, to determine their bioavailability from foods and mixed diets, to investigate their biological activities in cancer prevention, in bone and joint health, in cardiovascular health, and in physiological and psychological development and function.

1b. Approach (from AD-416)

1. Provide expert guidance and technical support for human studies to elucidate functions of and quantitative needs for nutrients in maintaining health of adults through reduction of risk factors for cardiovascular disease, diabetes, cancer, osteoporosis and other degenerative diseases.
2. Plan and conduct human studies (including residential, non-residential and field-based investigations) using such approaches as dietary recall, metabolic balance, radio/stable isotope retention, physiological/neurological function assessment, and specific metabolic/enzyme analyses.
3. Design diets to contain known amounts of essential and non-essential nutrients, or foods containing specific nutrients or other bioactive components.
4. Recruit, interview and screen volunteers for eligibility in human studies.
5. Determine clinical chemical (blood constituents), physiological (blood pressure, cardiovascular function, respiratory function, neuro-muscular function), neurological (mood, neurologic function), urinary and fecal excretion, and other measures of biological activity and health status.
6. Publish scientific results.

3. Progress Report

This report documents research conducted under a specific cooperative agreement between ARS and the University of North Dakota. Additional details of research can be found in the report for the parent project 5450-51000-035-00D, Mineral Utilization and Bioavailability in the 21st Century, with Changing Diets and Agricultural Practices. The project involved six (6) new studies, one (1) survey and data analysis of 13 previously completed studies.

Found dietary selenomethionine increases biomarkers of selenium status. We completed a 1-yr trial to determine the relationship of selenium status and level of supplementation with a food form of selenium. Plasma selenium level responded linearly to increases in dietary selenomethionine intake adjusted for metabolic body size, which

Project Number: 5450-51000-035-18S

Accession: 0407722

FY: 2008

we modeled. IMPACT: This algorithm will enable calculating the amount of dietary selenium needed to support selenium status for minimal cancer risk. This approach is needed to estimate the value of food sources of selenium in cancer prevention. NP107 Human Nutrition: Component 6. Prevention of obesity and disease: relationship between diet, genetics, and lifestyle.

Found a high meat-protein diet with high potential acid load does not impair calcium retention in women. IMPACT: This study confirms previous work at the Center; it shows that diets high in meat protein do not impair the use of dietary calcium in healthy adults and refutes previous results with purified proteins in rats. NP107 Human Nutrition: Component 4. Nutritional Requirements.

Found high bioavailability diets allow humans to adaptively increase their zinc absorption in response to low zinc intakes. Results gave a model to predict zinc absorption from dietary zinc and phytic acid. IMPACT: These results will assist in setting dietary zinc recommendations, which are based in part on predicted zinc absorption. NP107 Human Nutrition: Component 2: Bioavailability of Nutrients and Food Components, and Component 4. Nutritional Requirements.

Found in a state-wide survey of 246 charitable food pantries and their users that the system served 8% of the ND population. User demand increases particularly among older and working-poor and clients in rural areas. IMPACT: The food pantry system may be an appropriate modality for health-based interventions. NP107 Human Nutrition: Component 6. Prevention of obesity and disease: relationship between diet, genetics, and lifestyle.

Found regular physical activity induces beneficial changes in skeletal muscle that are limited by low copper intake. IMPACT: Individuals regularly engaging in exercise should ensure adequate copper intake. NP107 Human Nutrition: Component 6. Prevention of obesity and disease: relationship between diet, genetics, and lifestyle.

Conducted pilot program of healthy food choices and exercise for 311 children enrolled in summer programs. This pilot showed the potential for nesting intervention within such a program. NP107 Human Nutrition: Component 6. Prevention of obesity and disease: relationship between diet, genetics, and lifestyle.

Screened 415 candidates, enrolling 92 in a study to determine the effects of magnesium supplementation on magnesium status and sleep pattern. NP107 Human Nutrition: Component 6. Prevention of obesity and disease: relationship between diet, genetics, and lifestyle.

ADODR monitoring included on-site visits and technical guidance, conference calls and meetings, e-mail and telephone communications.

4. Accomplishments:

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/29/2008

Project Number: 5450-51000-035-22T

Accession: 0408646

FY: 2008

ModeCode: 5450-20-00 NORTHERN PLAINS AREA

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Prin Invs: JAY J CAO

Start Date: 05/01/2004

Term Date: 04/30/2009

National Programs: 107 N Human Nutrition

Title: HIGH SELENIUM PINTO BEANS AS A VALUE-ADDED PRODUCT

Period Covered From: 10/2007 To: 9 / 2008

Final Report? No

Terminate in Two Months? No

Agreement Number: 58-5450-4-0418

Organization Name: NORTHARVEST BEAN GROWERS ASSOCIATION

Progress and Outcomes:

1a. Objectives (from AD-416)

Determine the selenium content of pinto beans. Determine factors that increase the concentration of selenium in pinto beans. Use GIS data base to model mineral accumulation in pinto beans.

1b. Approach (from AD-416)

Pinto bean samples will be obtained at the time of harvest directly from producers in central North Dakota. Beans will be analyzed for minerals including selenium. Data on mineral content will be combined with producer information and data from the GIS data base. These data will be modeled by statistical methods to determine factors that result in selenium accumulation in pinto beans.

3. Progress Report

This report serves to document research conducted under a Trust Fund Cooperative Agreement between ARS and Northarvest Bean Growers Association. Details of this project can be found in the report for the parent CRIS 5450-510000-039-00D, MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES

The purpose of this agreement was to determine factors that affect the accumulation of selenium in pinto beans. Dry edible beans are a major crop raised in North Dakota, and there may be substantial health benefits associated with consumption of beans. With a change in personnel on this project, studies were re-designed and initiated to evaluate the effects of selenium and other antioxidants in beans on bone metabolism in a mouse model and on the cellular functions of bone-forming (osteoblasts) and bone-remodeling (osteoclasts) cells in a cell culture system.

An experiment was performed to determine whether bean hull extract supplementation slows age-related bone loss and osteoclast function. Tibias have been collected and will be evaluated with μ CT. The bean hull extract supplementation decreased osteoclast numbers in cultured bone marrow cells and mRNA levels of tartrate-resistant acidic phosphatase, a marker of osteoclast activity, in cultured osteoclasts. Another experiment is currently underway to determine whether long-term bean hull extract supplementation affects bone structure of femurs and osteoblast function.

ADODR monitoring activities to evaluate and discuss the research progress included

Project Number: 5450-51000-035-22T

Accession: 0408646

FY: 2008

teleconferences and electronic mail correspondence.

4. Accomplishments:

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/23/2008

Project Number: 5450-51000-036-00D

Accession: 0408616

FY: 2008

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: DAVID M KLURFELD

Prin Invs: ERIC O UTHUS

Start Date: 07/21/2004

Term Date: 04/30/2009

National Programs: 107 N Human Nutrition

Title: ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN
CANCER RISK

Period Covered From: 10/2007 To: 9 /2008

Final Report? No

Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Determine the molecular and cellular mechanism(s) of action of selenium (Se) in anti-carcinogenesis. Specific objectives include 1) Determine the role of Se in cell cycle progression and apoptosis in models of colon cancer; 2) Determine the role of selenoproteins in cancer prevention and the role of dietary components in the regulation of selenoprotein activity; 3) Determine the mechanism(s) by which Se alters DNA methylation and 4) Determine the relationship of oral selenium intake with selenium status and indicators of cancer risk.

1b. Approach (from AD-416)

A variety of cell culture and animal model approaches will be used. In general, cell culture experiments will be run using cell lines specific for colon. Various forms and concentrations of selenium will be added to serum-free media. Cell growth, indices of selenium status, and indices of cell cycle progression and apoptosis will be measured. These studies will be used to determine the effects of nutritional levels of selenium in supporting cellular survival signaling in human cultured colon cells, and the role of the putative anti-tumorigenic selenium-metabolite, methylselenol, in cell cycle progression and apoptosis in human cultured colon cells. Other cell culture models (colon and/or liver cell lines) will be used in siRNA knockdown studies. These experiments will determine the effect of selenium in cells in which specific genes have been knocked down by siRNA. Other studies will use knock downs of various selenoproteins to determine their role in anticarcinogenicity of selenium. Animal studies will use rats and mice to determine the effects of form and concentration of dietary selenium on 1) selenoprotein expression and activity as related to carcinogenesis, 2) carcinogen-induced aberrant crypt formation (preneoplastic colon cells) and, 3) indices of oxidative stress and one-carbon metabolism including DNA methylation of genomic and gene specific DNA.

2. Milestones for FY2008

1. Finish cell culture experiments designed to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway.
Milestone Fully Met
2. Finish cell culture experiments designed to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells.
Milestone Fully Met
3. Initiate studies to determine the role of differentially expressed genes, as

Project Number: 5450-51000-036-00D

Accession: 0408616

FY: 2008

discovered in previous cell culture studies, in mediating the anti-tumorigenic effect of selenium

Milestone Fully Met

4. Complete preparation of high-selenium soy protein for experimental use: obtain newly harvested high-selenium soybeans, complete selenium analysis on the soybeans, complete soy protein isolation.

Milestone Fully Met

5. Complete preparation of high-selenium wheat flour for experimental use: obtain high-selenium wheat; complete selenium analysis on the wheat; complete processing to whole wheat flour.

Milestone Fully Met

6. Establish animal models of metastasis (experimental and spontaneous models) to be used in selenium studies.

Milestone Fully Met

7. Initiate RLGS study in Ames mice fed various forms of selenium.

Milestone Substantially Met

RLGS is no longer the method of choice for determination of DNA promoter region methylation - commercially available methylation arrays are now being used. The initial experiment, which is underway, is using mice fed chow diets. After validation of the methylation arrays, mice fed selenium will be used.

8. Complete analyses from the Ames dwarf mouse selenium study.

Milestone Not Met

Other

see #7 above; study will proceed following validation of methylation arrays

9. Determine whether there is a relationship between methionine sulfoxide activity and/or expression and aberrant crypt formation in and aberrant crypt model (contingency for 2.6)

Milestone Substantially Met

work will be completed early FY 2009

10. Complete validation of HPLC-ICP-MS analytical procedure and report results

Milestone Not Met

Other

We determined that while ICP-MS technology is sufficient for detecting Se, this approach depends on presumptive identification of Se-metabolites based on co-elution with known standards, thus limiting its use to those Se-species that can be obtained in pure form. We are acquiring a Q-instrument, which will enable us to use metabolomic approaches in identifying all major Se-metabolites, including the selenoproteins in plasma. We anticipate completing this work in FY 2009

11. Complete selenium intervention trial; continue associated analytical work.

Milestone Fully Met

3 . Progress Report

A number of studies designed to determine the mechanism(s) of chemoprevention by the nutrient selenium have been undertaken - accomplishments from those studies are listed below. All current work is being undertaken to finish work as proposed in this CRIS project. This includes work on data collection and bioinformatics on genes that are important in the cancer/anticancer process (as affected by selenium). These genes were discovered in previous experiments done by cell culture and animal studies at the Center. Studies to determine the correlation of the activity of methionine sulfoxide

Project Number: 5450-51000-036-00D

Accession: 0408616

FY: 2008

reductase, a selenoprotein important in antioxidant defense, to genes affected in an animal carcinogen model are underway. The analytical work for the LoDoSe selenium intervention study is being completed. This includes work on gene polymorphisms that relates selenium intake to selenium status or biomarkers of selenium status. Analytical method development to assess selenium status is still underway. This work will enable correlating selenium intake to status assessment variables and ultimately to health outcome. A new 5-year plan entitled "Mechanisms of Cancer Prevention by Selenium" for 2.6 SY has been written and is now being reviewed by OSQR. (NP107, Component 6: Prevention of Obesity and Disease: Relationship between Diet, Genetics, and Lifestyle.)

4. Accomplishments:

1 Dietary induced obesity enhances secondary tumor formation.

Diet is the single greatest contributor to human cancer, possibly accounting for one-third of the disease. While a number of dietary carcinogens have been identified, the primary risk from diet is thought to be as a result of non-optimal intakes of specific food components known to inhibit cancer. Furthermore, excess intake of specific food components can increase the risk of certain cancers. We now know that, obesity is fast becoming a leading risk factor for cancer. We have successfully established animal models that can be used to study the impact of diet on secondary cancer prevention. By using these models we demonstrated that dietary induced obesity enhanced secondary tumor development and tumor growth. IMPACT: These successes will lead to studies on dietary intervention and prevention of obesity-related secondary cancer. NP107, Component 6. Prevention of Obesity and Disease: Relationship between Diet, Genetics, and Lifestyle.

2 The selenium metabolite methylselenol may inhibit the growth of deoxycholic acid-resistant colon cells.

A typical part of the Western diet is a high fat intake that leads to increased level of fecal bile acids, and these bile acids, primarily deoxycholic acid (DCA) in humans are believed to be tumor promoters of colon cancer. It has been suggested that cell growth inhibition induced by DCA may cause compensatory hyperproliferation of colonic epithelial cells and provide selection for subpopulations of cells resistant to DCA's inhibitory effect. These surviving cells are somehow mutated and have a survival phenotype that is resistant to normal cell cycle arrest and apoptosis, and consequently increase the risk of mutations. On the other hand, there is increasing evidence for the efficacy of certain forms of selenium as cancer-chemopreventive compounds. Methylselenol has been hypothesized to be a critical selenium metabolite for anticancer activity in vivo. In this study, we demonstrated that, although both DCA and methylselenol inhibited colon cancer cell proliferation, DCA and methylselenol each used distinct molecular signaling pathways. Thus, our data provide, for the first time, the molecular basis for the inhibition of growth of deoxycholic acid-resistant colon cells by methylselenol. IMPACT: The study of comparative effects of methylselenol and DCA on cell proliferation and signaling pathways will further our understanding of functional role of methylselenol as a cancer-chemopreventive compound as related to a high-fat diet. NP107: Component 6. Prevention of Obesity and Disease: Relationship between Diet, Genetics, and Lifestyle.

3 Dietary copper and selenium interact to affect the concentration of plasma homocysteine.

Previously we reported that copper deficiency decreased plasma homocysteine and increased plasma glutathione in rats. Other work that we have done shows that selenium deficiency also decreases plasma homocysteine and increases plasma glutathione in rats. We suggested a common mechanism in each deficiency - an up-

Project Number: 5450-51000-036-00D

Accession: 0408616

FY: 2008

regulation in the enzyme that makes glutathione from cysteine, a metabolite of homocysteine. Our findings showed that a combined deficiency of both copper and selenium did result in significantly lower plasma homocysteine and significantly elevated plasma glutathione. Studies are ongoing to determine whether marginal deficiencies have synergistic effects on plasma homocysteine and glutathione. IMPACT: Plasma homocysteine is a biomarker of cardiovascular disease. Understanding dietary factors that affect the plasma concentration of homocysteine are needed to validate its impact as a biomarker. NP107: Component 6. Prevention of Obesity and Disease: Relationship between Diet, Genetics, and Lifestyle.

- 4 Dietary selenomethionine increases biomarkers of selenium status in a predictable way. While selenium supplementation has been shown to reduce cancer risk in both animals and humans, the minimal effective dose has not been established for humans as all clinical trials have used the same dose, 200 mcg/day. Our previous studies indicate that lower doses are likely to be effective, namely, doses that support plasma selenium concentrations of ca. 120 ng/ml. We completed a 1-yr randomized, multi-dose clinical trial to determine the relationship of selenium status as assessed by plasma, buccal cell and urinary selenium levels on baseline selenium status and level of supplementation with a food form of selenium. Our results showed that plasma selenium level (Y, in ng/ml) responds linearly to increases in dietary selenomethionine intake adjusted for metabolic body size (X, in mcg Se/d/kg^{0.75}) according to the function $Y = 18.2 X$. This study also yielded useful information regarding the predictive value of food frequency questionnaire methodology in estimating selenium intakes and status. IMPACT: This algorithm will facilitate calculating the amount of dietary selenium required to support selenium status associated with minimal cancer risk. This approach is required to understand the value of food sources of selenium in cancer prevention. NP107: Component 6. Prevention of Obesity and Disease: Relationship between Diet, Genetics, and Lifestyle.

5. Significant Activities that Support Special Target Populations

None

6. Technology Transfer

- 0 Number of New CRADAs
- 0 Number of Active CRADAs
- 1 Number of New MTAs (providing only)
- 0 Number of Invention Disclosures Submitted
- 0 Number of Patent Applications Filed
- 0 Number of New Germplasm Releases
- 0 Number of new commercial licenses granted
- 0 Number of web sites managed
- 6 Number of non-peer reviewed presentations and proceedings
- 2 Number of newspaper articles and other presentations for non-science audiences
- 0 Number of Other Technology

New MTA (providing only) Details:

01 MTA ID: 5296

PCNA promoter-luciferase construct

Technology: MTA for developing wheat varieties for organic agriculture

Project Number: 5450-51000-036-00D

Accession: 0408616

FY: 2008

Transfer: mineral analysis on various wheat varieties
Customer/User: Dr. Jones, Washington State University
Impact/Outcome: this MTA will be used to determine mineral content in various wheat samples; the information will be used in developing more nutrient dense wheat varieties.

Non Peer Reviewed Presentations and Proceedings Details:

- 01 **Description:** Technology: High dietary intake of sodium selenite does not affect gene mutation frequency in rat colon and liver
Transfer: Oral presentation given at Experimental Biology annual meeting (Zeng, H., Uthus, E.O., Ross, S.A., Davis, C.D. High dietary intake of sodium selenite does not affect gene mutation frequency in rat colon and liver. Experimental Biology annual meeting, San Diego, CA, April 5-9, 2008, Faseb J 22:146.7)
Customer/User: Other scientists
Impact/outcome: expand understanding of the role of selenium as a chemopreventive nutrient.
- 02 **Description:** Technology: Selenium is critical for the regulation of tumor suppressor and pro-inflammatory gene expression in human colon Caco-2 cells
Transfer: Oral presentation given at Experimental Biology annual meeting (Zeng, H., Botnen, J.H. Selenium is critical for the regulation of tumor suppressor and pro-inflammatory gene expression in human colon Caco-2 cells. Experimental Biology annual meeting, San Diego, CA, April 5-9, 2008, Faseb J 22:1124.6)
Customer/User: Other scientists
Impact/outcome: expand understanding of the role of selenium as a chemopreventive nutrient.
- 03 **Description:** Technology: Dietary selenium (Se) and copper (Cu) interact to affect homocysteine metabolism in rats
Transfer: Poster presentation given at Experimental Biology annual meeting (Uthus, E.O., Ross, S.A. Dietary selenium (Se) and copper (Cu) interact to affect homocysteine metabolism in rats. Experimental Biology annual meeting, San Diego, CA, April 5-9, 2008, Faseb J 22:696.3)
Customer/User: Other scientists
Impact/outcome: expand understanding of the role of selenium as a chemopreventive nutrient.
- 04 **Description:** Technology: Absence of diabetes indicators in a selenium-supplementation trial
Transfer: Poster presentation given at Experimental Biology annual meeting (Combs, G.F., Watts, J.C., Johnson, L.K., Canfield, W.K., Davis, C.D., Milner, J.A. Absence of diabetes indicators in a selenium-supplementation trial. Experimental Biology annual meeting, San Diego, CA, April 5-9, 2008, Faseb J 22:696.4)
Customer/User: Other scientists
Impact/outcome: expand understanding of the role of selenium as a

Project Number: 5450-51000-036-00D

Accession: 0408616

FY: 2008

chemopreventive nutrient.

- 05 **Description:** Technology: Responses to selenium supplementation in healthy Americans
Transfer: Oral presentation given at Experimental Biology annual meeting (Combs, G.F., Watts, J.C., Johnson, L.K., Canfield, W.K., Davis, C.D., Milner, J.A. Responses to selenium supplementation in healthy Americans. Experimental Biology annual meeting, San Diego, CA, April 5-9, 2008, Faseb J 22:146.2)
Customer/User: Other scientists
Impact/outcome: expand understanding of the role of selenium as a chemopreventive nutrient.
- 06 **Description:** Technology: More is not always better: using the dog model to identify what dose of selenium provides the best protection against prostate cancer
Transfer: Oral presentation given at Experimental Biology annual meeting (Waters, D.J., Shen, S., Kengeri, S., Chiang, E.C., Combs, G.F., Jr, Morris, J.S, Bostwick, D.G. More is not always better: using the dog model to identify what dose of selenium provides the best protection against prostate cancer. Experimental Biology annual meeting, San Diego, CA, April 5-9, 2008, Faseb J 22:146.8)
Customer/User: Other scientists
Impact/outcome: expand understanding of the role of selenium as a chemopreventive nutrient.

Newspaper Articles and Other Presentations for Non Science Audiences Details:

- 01 **Description:** Technology: Role of nutrition in cancer prevention.
Transfer: Newspaper
Grand Forks Herald, February 2008
Customer/User: General public
Impact/Outcome: Provide information for better nutritional health
- 02 **Description:** Technology: Role of nutrition in cancer prevention.
Transfer: Newspaper
Grand Forks Herald, May 2008
Customer/User: General public
Impact/Outcome: Provide information for better nutritional health

7. International Cooperation / Collaboration**Scientific Publications:**

Log 115

1. Clifford, A.J., De Moura, F.F., Ho, C.C., Chuang, J.C., Follett, J.R., Fadel, J.G., Novotny Dura, J. 2006. A feasibility study to quantify in vivo human alpha-tocopherol metabolism. American Journal of Clinical Nutrition. 84(6):1430-1441. 000022216
2. Combs, G.F. 2008. Leading issues in nutrition: What opinion leaders say. Nutrition Today. 43(2):82-84. 000021427

Project Number: 5450-51000-036-00D

Accession: 0408616

FY: 2008

3. Combs, G.F., Hassan, N., Dellagana, N., Stabb, D., Fischer, P., Hunt, C., Watts, J.J. 2008. Apparent efficacy of food-based calcium supplementation in preventing rickets in bangladesh. Biological Trace Element Research. 121:193-204. 000019588
4. Taylor, J.B., Marchello, M.J., Finley, J.W., Neville, T.L., Combs, G.F., Caton, J.S. 2008. Nutritive value and display-life attributes of selenium-enriched beef-muscle foods. Journal of Food Composition and Analysis. 21(2):183-186. 000020578
5. Waters, D.J., Shen, S., Xu, H., Kengeri, S.S., Colley, D.M., Chiang, E.C., Chen, Y., Schlittler, D., Oteham, C., Combs, G.F. 2007. Noninvasive prediction of prostatic DNA damage by oxidative stress challenge of peripheral blood lymphocytes. Cancer Epidemiology Biomarkers and Prevention. 16:1906-1910. 000020840
6. Zeng, H., Combs, G.F. 2008. Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasion. Journal of Nutritional Biochemistry. 19:1-7. 000020553

Approved: MCGUIRE MICHAEL R

Date: 09/17/2008

Project Number: 5450-51000-036-02R

Accession: 0410110

FY: 2008

ModeCode: 5450-20-00 NORTHERN PLAINS AREA

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: DAVID M KLURFELD

Prin Invs: GERALD F COMBS

Start Date: 09/01/2005

Term Date: 08/31/2010

National Programs: 107 N Human Nutrition

Title: SELENIUM NUTRITION IN HUMANS: PREDICTING DIETARY SELENIUM NEEDS TO ACHIEVE TARGET
BLOOD SELENIUM LEVELS

Period Covered From: 10/2007 To: 9 / 2008

Final Report? No

Terminate in Two Months? No

Agreement Number: 05-5450-5-0330

Organization Name: NATIONAL CANCER INSTITUTE, DEPARTMENT OF HEALTH AND HUMAN SERVICES,
NATIONAL INSTITUTES OF HEALTH

Progress and Outcomes:

1a. Objectives (from AD-416)

Develop algorithm relating increase in stable plasma Se level to that at baseline and level of supplemental Se.

1b. Approach (from AD-416)

Conduct a randomized, double-blind, intervention study will be conducted with healthy men (120) and women (120) randomized to 0, 50, 100, or 200 ug Se/day (as L-selenomethionine) administered in daily oral doses. Fasting blood samples and urine samples will be drawn two wks prior to and periodically throughout the 1-yr study. The following measurements will be made: Se, homocysteine, vitamin B12 and folate in plasma; Se and 8a-deoxyquanosine in urine; DNA damage and allelic variants of Se-dependent enzymes in lymphocytes. Results will be used to compute the relationship of final-plateau plasma (9-12 mos.) Se concentration as a function of baseline (0 mos.) Se level, Se dose, metabolic body size and urinary Se, as well as outcomes related to carcinogenesis.

3. Progress Report

This report serves to document research conducted under an interagency reimbursable agreement between ARS and National Cancer Institute of the National Institutes of Health. Additional details of research can be found in the report for the parent project 5450-51000-036-00D, ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK

This collaboration planned and implemented the first clinical intervention trial using nutritional doses of selenium, the "LoDoSe trial", the intervention phase of which was initiated in FY2006 and was completed in FY2008. This trial will provide the fundamental information to allow the projection of daily dietary selenium needs from plasma selenium levels found to be thresholds for such health effects as minimalization of cancer risks in healthy Americans, which prospect has direct implications to the U.S. food industry which can produce foods capable of providing 50-100 mcg selenium per day through geographic sourcing, or selenium-fortification. This year, 259 adult men and women volunteers participated in and completed the year-long intervention. Studies of the baseline characteristics of the cohort showed that: i) The cohort was relatively high in selenium status, with average plasma selenium level, 142 ppb, greater than that of the NHANES-III cohort; ii) plasma selenium level was positively associated with

Project Number: 5450-51000-036-02R

Accession: 0410110

FY: 2008

buccal cell selenium content (validating buccal cells as a biomarker of selenium status); iii) plasma selenium was not related to risk to type 2 diabetes (refuting suggestions based on secondary analyses of other cohorts); gender and selenium-dependent glutathione peroxidase genotype were significant determinants of the 1-yr responses of urinary selenium level to oral selenium dose, but not to the corresponding increases in plasma selenium (suggesting differences in selenium metabolism among these groups which differ in cancer risk).

4. Accomplishments:**7. International Cooperation / Collaboration****Scientific Publications:**

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/23/2008

Project Number: 5450-51000-036-03N Accession: 0412134 FY: 2008

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: DAVID M KLURFELD

Prin Invs: LIN YAN

Start Date: 08/01/2007

Term Date: 12/31/2011

National Programs: 107 N Human Nutrition

Title: ANTICANCER EFFECTS OF HIGH-SELENIUM SOYBEANS

Period Covered From: 10 / 2007 To: 9 / 2008

Final Report? No

Terminate in Two Months? No

Agreement Number: 58-5450-7-0119N

Organization Name: UNIV OF NEBRASKA

Progress and Outcomes:

1a. Objectives (from AD-416)

To investigate whether high-selenium soybeans have greater anticancer effects that low-selenium soybeans in animal models.

1b. Approach (from AD-416)

UNL will be responsible for identifying an appropriate line of soybeans (based on its nutrient contents) and determining the stages of the plant development that are most appropriate for selenium fertilization. UNL will be responsible for planting, fertilizing the plants with selenium, harvesting and providing the beans to GFHNRC.

GFHNRC will be responsible for designing and conducting animal studies. This includes dietary preparation, feeding animals, carcinogen treatment, monitoring the progress, collecting and analyzing data.

GFHNRC and UNL together will interpret data, draw conclusions from the investigation and prepare manuscripts for publication in scientific journals.

3. Progress Report

This report serves to document research conducted under a Non-Funded Cooperative Agreement between ARS and the University of Nebraska-Lincoln. Additional details of research can be found in the report for the parent project 5450-51000-036-00D, ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK

We produced a small quantity of high-selenium soybean seeds from 2007 crop year, processed the protein isolate and completed a feeding experiment to assess selenium bioavailability. We planted soybeans in Agronomy Research Farm at University of Nebraska-Lincoln in 2008 crop year. Upon harvest in October, if the seeds reach the targeted selenium level, we will have it processed to protein isolate for research use.

ADODR monitoring activities included phone calls, e-mails, conference calls, and meetings at scientific venues.

4. Accomplishments:

01/09/2009

Agricultural Research Information System
Report of Progress (AD-421)

Page: 57

Project Number: 5450-51000-036-03N

Accession: 0412134

FY: 2008

7. International Cooperation / Collaboration

Scientific Publications:

Log 115

Approved: MCGUIRE MICHAEL R

Date: 09/23/2008

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